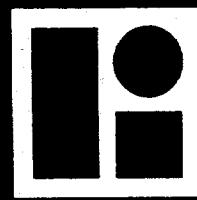


Final report-Mutagenicity Evaluation of Sodium Ascorbate U. S. P., F. C. C.

FDA 75-64

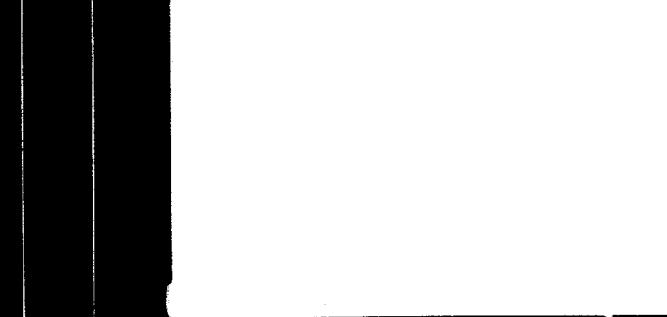
10/29/76

1EE



**Litton**

**BIONETICS**



5516 Nicholson Lane  
Kensington, Maryland  
20795

MUTAGENICITY EVALUATION  
OF  
SODIUM ASCORBATE U.S.P., F.C.C.  
FDA 75-64

FINAL REPORT

SUBMITTED TO

FOOD AND DRUG ADMINISTRATION  
DEPARTMENT OF HEALTH, EDUCATION AND WELFARE  
5600 FISHERS LANE  
ROCKVILLE, MARYLAND

SUBMITTED BY

LITTON BIONETICS, INC.  
5516 NICHOLSON LANE  
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 2672

OCTOBER 29, 1976



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EVALUATION SUMMARY

The test compound Sodium Ascorbate U.S.P., F.C.C., FDA 75-64, 000134-03-2, did not exhibit mutagenic activity in any of the assays employed in these studies.

DATE: October 29, 1976

SPONSOR: U.S. Food and Drug Administration

SUBJECT: Evaluation of Test Compound Sodium Ascorbate U.S.P., F.C.C., FDA 75-64

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: September 3, 1976
2. Description: white powder

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain:	<u>Saccharomyces cerevisiae</u> , strain D4
Bacteria Strains:	<u>Salmonella typhimurium</u> , strains TA-1535
	TA-1537
	TA-1538
	TA-98
	TA-100

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

<u>Component</u>	<u>Final Concentration/ml</u>
1. TPN (sodium salt)	4 $\mu$ moles
2. Glucose-6-Phosphate	5 $\mu$ moles
3. Sodium Phosphate (dibasic) pH 7.4	100 $\mu$ moles
4. $MgCl_2$	8 $\mu$ moles
5. $KCl$	33 $\mu$ moles
6. Homogenate fraction equivalent to 25 mg of wet tissue.	



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#### D. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from tissues of the following mammalian species: Mouse - ICR random bred adult males; rat - Sprague-Dawley adult males; and monkey - Macaca mulatta adult males.

#### E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1  
POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

<u>Assay</u>	<u>Chemical<sup>a</sup></u>	<u>Solvent</u>	<u>Probable Mutagenic Specificity</u>
Nonactivation	Methylnitrosoguanidine	Water or saline	BPS <sup>b</sup>
	Ethylmethanesulfonate	Water or saline	BPS <sup>b</sup>
	2-Nitrofluorene	Dimethylsulfoxide <sup>c</sup>	FS <sup>b</sup>
	Quinacrine mustard	Water or saline	FS
Activation	Dimethylnitrosamine	Water or saline	BPS <sup>b</sup>
	2-Acetylaminofluorene	Dimethylsulfoxide <sup>c</sup>	FS <sup>b</sup>
	8-Aminoquinoline	Dimethylsulfoxide <sup>c</sup>	FS <sup>b</sup>
	2-Aminoanthracene	Dimethylsulfoxide <sup>c</sup>	BPS <sup>b</sup>

<sup>a</sup> Concentrations given in the Results Section

<sup>b</sup> BPS = base-pair substitution; FS = frameshift

<sup>c</sup> Previously shown to be non-mutagenic

### III. METHODS

#### A. Toxicity

The solubility, toxicity and doses for the test chemical were determined prior to screening.

The test chemical was tested for toxicity against specific indicator strains over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival concentrations and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for the chemical with a given strain, then a maximum dose of 5% (w/v) was used.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.



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## B. Plate Tests (Overlay Method)

Approximately  $10^8$  cells from an overnight culture of each indicator strain were added to test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For nonactivation tests, the three dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests 0.5 ml of a 9,000 x g tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. Three dose levels of the test chemical were added to the appropriate tubes, which were then mixed and the contents poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. The plates were incubated for 48 to 72 hours at 37°C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using positive compounds that are active directly and those that require metabolic activation were run with each assay.

## C. Suspension Tests

### 1. Nonactivation

Bacteria and yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of  $1 \times 10^{10}$  cells/ml and  $5 \times 10^9$  cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic, 24-well tissue culture plates (Linbro). Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.5 ml. The solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a  $10^{-1}$  dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

### 2. Activation

Bacteria and yeast cells were grown and prepared as described in the nonactivation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for nonactivation tests.



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D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (except monkeys) sufficient to provide the necessary quantities of tissues were killed by cranial blow, decapitated and bled. Monkey tissues were obtained from freshly killed and bled male rhesus monkeys. Organs were immediately dissected from the animals using aseptic techniques and placed in ice-cold 0.15 M KCl. Upon collection of the desired quantity of organs, they were washed twice with fresh KCl and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies. Protein and P-448 determinations were made for each lot of homogenate.

E. Data Recording and Reporting

1. Suspension assays

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. The data were then processed and printed from a computer program. All raw data sheets are dated and signed by the responsible technician.

2. Plate test assays

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were entered into a computer program designed to print out all data by test. The data are presented as revertants per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points.

IV. RESULTS SECTION

A. Solubility Properties of the Test Compound

1. Name or code designation of the test compound: 000134-03-2
2. Test solvent: Saline
3. Solubility of the test compound under treatment conditions:  
Soluble
4. Additional comments: White powder

B. Toxicity and Dosage Determinations for the Test Compound

1. Test date for toxicity determination: September 8, 1976
2. The 50% survival level was determined for bacteria and yeast indicator organisms by conducting survival curves with the test compound at the following concentrations:

Percent Concentration (w/v or v/v)

5.0  
0.5  
0.05  
0.005  
0.0005

3. Concentrations of the test compound used in the mutagenicity tests:

<u>Test Doses</u>	<u>Percent Concentration</u>	
	<u>Bacteria</u>	<u>Yeast</u>
1/4 50% Survival	0.075	1.25
1/2 50% Survival	0.150	2.50
50% Survival	0.100	5.00

C. Suspension Assay Results

The suspension test results for the test compound are summarized in the following six tables. The values presented in these tables are the calculated mutation frequencies for each control and experimental test point. The first table of the suspension set presents the results for the nonactivation assays, and the second through the fourth table of the suspension set presents the results for the activation assays. The fifth table shows the results of the nonactivation plate test and the sixth table shows the results of the activation plate test. A listing of computer codes and abbreviations is included for reference. Tabulation of all raw data is provided in the Appendix.



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DATA TABLE TERMS AND ABBREVIATIONS

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION																														
COMPOUND	Client designated compound number appears in this column.																														
TEST CODES	<table> <tr> <td>NAN</td><td>= Nonactivation: Solvent Control</td></tr> <tr> <td>NAP</td><td>= Nonactivation: Positive Control</td></tr> <tr> <td>NA1</td><td>= Nonactivation: Test Compound Dose 1</td></tr> <tr> <td>NA2, etc.</td><td>= Reflects the other dose level(s)</td></tr> <tr> <td>A+C</td><td>= Negative Chemical Control for ACP</td></tr> <tr> <td>A-C</td><td>= Activation: Solvent Control</td></tr> <tr> <td>ALI</td><td>= Activation: Homogenate Control (Liver)</td></tr> <tr> <td>ALU</td><td>= Activation: Homogenate Control (Lung)</td></tr> <tr> <td>ACP</td><td>= Activation: Positive Control</td></tr> <tr> <td>ACT</td><td>= Activation Test</td></tr> <tr> <td>LI</td><td>= Liver Tissue Activation Fraction</td></tr> <tr> <td>LU</td><td>= Lung Tissue Activation Fraction</td></tr> <tr> <td>KI</td><td>= Kidney Tissue Activation Fraction</td></tr> <tr> <td>TE</td><td>= Testes Tissue Activation Fraction</td></tr> <tr> <td>1,2, etc.</td><td>= Dose Levels</td></tr> </table>	NAN	= Nonactivation: Solvent Control	NAP	= Nonactivation: Positive Control	NA1	= Nonactivation: Test Compound Dose 1	NA2, etc.	= Reflects the other dose level(s)	A+C	= Negative Chemical Control for ACP	A-C	= Activation: Solvent Control	ALI	= Activation: Homogenate Control (Liver)	ALU	= Activation: Homogenate Control (Lung)	ACP	= Activation: Positive Control	ACT	= Activation Test	LI	= Liver Tissue Activation Fraction	LU	= Lung Tissue Activation Fraction	KI	= Kidney Tissue Activation Fraction	TE	= Testes Tissue Activation Fraction	1,2, etc.	= Dose Levels
NAN	= Nonactivation: Solvent Control																														
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LU	= Lung Tissue Activation Fraction																														
KI	= Kidney Tissue Activation Fraction																														
TE	= Testes Tissue Activation Fraction																														
1,2, etc.	= Dose Levels																														
CONCENTRATION	<p>All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units.</p> <p>Example: 0025-2PCT = 0.25 percent concentration</p>																														
POPU	Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (i.e., EP + 6 = $\times 10^6$ ).																														
MUT 1	Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (i.e., EP + 0 = $10^0$ ). For strain D4, MUT 1 represents the number of ADE+ convertants.																														
MUT 2	Only used for strain D4 and represents the number of TRY+ convertants in the plated sample.																														
FREQ 1	The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE+ value.																														
FREQ 2	Only used for strain D4 and represents the TRY+ conversion frequency.																														
CONTAM	Presence of contamination on any plates.																														

DATA TABLE TERMS AND ABBREVIATIONS (continued)

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
AAF	2-Acetylaminofluorene
DMSO	Dimethylsulfoxide
DMN	Dimethylnitrosamine
EMS	Ethylmethanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
ANTH	2-Amino Anthracene
AMQ	8-Amino Quinoline
SPECIES	Animal Strains
SPRDAW	Sprague Dawley Rats
ICRFLO	Flow ICR Random Bred Mice
RHESUS	Rhesus Monkey ( <u>Macaca mulatta</u> )
MIXEDB	Dog, Mixed Breed
NEWZEA	New Zealand White Rabbit
UG	Microgram
UM	Micromole
ADE	Adenine
TRY	Tryptophan

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 10/27/76

SPECIES / NONACTIVATION COMPOUND 000134032

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	CONTROLS
NAN		66.63	18.29	14.48	1.21	4.11	13.86	9.92	22.36	9.75
NAP		729.93	4938.27	95.62	143.90		820.41		68.71	38.10
NA1		70.97	13.91	13.58	1.56	6.45	31.67	14.61	20.39	24.42
NA2		72.70	19.46	12.98	2.64		15.19		24.57	10.78
NA3		41.79	9.12	29.55	1.17		12.33		20.55	11.50

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 10/27/76

SPECIES ICHIFLO/MOUSE

COMPOUND 000134032

TEST	ORG	TA100 HIS Ex-B	TA1535 HIS Ex-B	TA1537 HIS Ex-B	TA1538 HIS Ex-B	TA98 HIS Ex-B	TA98 HIS Ex-B	0000D4 ADE Ex-5	0000D4 TRY Ex-5
ACT	A+C	20.58	7.58	2.69	29.04	4.72		23.27	8.14
ACT	A-C	22.30	5.57	5.85	28.54	3.09		26.15	7.98
ACT	ALI	24.42	6.44	6.95	49.71	9.94		26.53	8.68
ACT	ALU	21.77	7.57	3.65	25.54	5.41		28.89	8.63
ACT	PLI	70.14	136.26	142.77	202.67	106.30		67.69	27.36
ACT	PLU	22.31	27.00	2.14	38.36	110.39		32.40	11.43
ACT	L11	23.25	7.92	7.87	13.52	9.74		21.59	11.38
ACT	L12	23.82	10.39	9.60	24.73	15.22		19.95	8.33
ACT	L13	24.95	14.63	6.48	34.04	19.63		26.72	7.34
ACT	L01	22.58	5.67	7.28	16.26	46.59	16.34	29.12	11.86
ACT	L02	23.62	5.67	8.19	23.95	13.21		26.05	8.56
ACT	L03	25.05	13.06	7.89	21.86	13.46		27.87	9.78

TEST DATA

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 10/27/76

SPECIES SPRDAW/RAT

COMPOUND 000134032

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 AOE EX-5	0000D4 AOE EX-5	TRY EX-5
ACT	A+C	20.72	10.78	4.17	4.92	15.89	43.00	21.56	
ACT	A-C	25.83	10.16	2.65	4.12	12.44	51.25	20.37	
ACT	ALI	31.45	13.54	3.74	18.45	11.62	41.81	26.41	NEGATIVE CONTROL
ACT	ALU	27.04	12.64	2.11	7.49	20.06	39.12	21.07	
ACT	PLI	61.73	246.86	122.50	218.59	84.05	79.89	60.49	
ACT	PLU	28.22	13.97	1.37	273.20	24.85	40.92	22.52	POSITIVE CONTROL
ACT	L11	31.96	16.32	2.99	14.75	22.07	38.35	14.81	
ACT	L12	40.07	15.36	2.70	16.87	26.10	43.20	17.73	
ACT	L13	35.80	12.97	2.91	6.98	24.36	49.10	21.93	
ACT	L01	58.10	5.58	2.03	16.67	27.29	39.52	13.66	TEST DATA
ACT	L02	32.72	4.45	2.09	13.10	19.07	41.15	14.19	
ACT	L03	34.71	6.01	1.94	8.22	20.01	48.69	15.88	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 10/27/76

SPECIES RHECUS/MONKEY COMPOUND 000134032

TEST	ORG	TA100 HIS Ex-8	TA1535 HIS Ex-8	TA1537 HIS Ex-8	TA98 HIS Ex-8	0000D4 ADE Ex-5	0000D4 TRY Ex-5
ACT	A+C	26.73	7.45	11.79	3.31	26.43	13.21
ACT	A-C	25.84	9.64	7.98	6.21	27.12	16.19
ACT	ALI	30.06	6.89	18.45	10.77	56.55	15.71
ACT	ALU	28.62	7.57	22.19	3.79	61.49	8.72
ACT	PLI	60.22	58.15	3.07	580.84	88.51	24.55
ACT	PLU	30.95	8.82	13.13	3.86	42.92	12.93
						6.12	POSITIVE CONTROL
ACT	L11	30.16	7.71	19.62	11.86	41.04	14.79
ACT	L12	32.04	9.63	20.46	4.55	27.46	16.82
ACT	L13	26.49	7.62	19.75	10.31	30.93	13.86
ACT	L01	30.91	6.92	24.47	17.27	39.66	12.13
ACT	L02	26.73	7.94	45.60	15.00	41.44	11.81
ACT	L03	29.11	6.83	23.47	5.00	34.91	11.39
						7.72	

## SUMMARY\_OF\_TEST\_RESULTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 000134032  
 B. TEST DATE: OCT. 12, 1976

## PLATE\_IESIS

TEST	SPECIES	ISSUE	PLATE_IESIS		PLATE	
			IA-1535	IA-1537	IA-1538	IA-28
1. NON-ACTIVATION SOLVENT CONTROL*			---	31	31	19
POSITIVE CONTROL**			---	>1000	>1000	>1000
TEST	0.30000 %			895	461	>1000
	0.15000 %			34	26	9
	0.07500 %			23	40	30
	0.037500 %			28	36	16
2. ACTIVATION SOLVENT CONTROL*			MOUSE	25	40	20
RAT	RAT		LIVER	20	20	14
MONKEY	MONKEY		LIVER	16	41	12
TEST	0.30000 %		LIVER	202	154	303
	0.15000 %		LIVER	94	91	>1000
	0.07500 %		LIVER	513	375	80
	0.037500 %		LIVER	21	26	25
	0.0187500 %		LIVER	27	35	26
	0.00937500 %		LIVER	39	54	15
	0.004687500 %		RAT	15	19	12
	0.0023437500 %		RAT	18	18	6
	0.00117187500 %		RAT	16	23	13
	0.000585937500 %		MONKEY	37	30	9
	0.0002929687500 %		MONKEY	31	40	13
	0.00014648437500 %		MONKEY	25	36	14

\* NON-ACTIVATION ASSAYS CONSIST OF THE CELLS PLUS THE TEST COMPOUND VEHICLE (SOLVENT). FOR ACTIVATION ASSAYS, THE OVERLAY CONTAINS THE ACTIVATION SYSTEM PLUS THE TEST COMPOUND VEHICLE.

\*\* TA-1535 MNNG 2 UG/PLATE  
 TA-1537 GM 20 UG/PLATE  
 TA-1538 NF 100 UG/PLATE  
 TA-98 NF 100 UG/PLATE  
 TA-100 MNNG 2 UG/PLATE  
 NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS(UL) OR MICROGRAMS(UG) PER PLATE.

\*\*\* TA-1535 ANTH 100 UG/PLATE  
 TA-1537 AMQ 100 UG/PLATE  
 TA-1538 AAF 100 UG/PLATE  
 TA-98 AAF 100 UG/PLATE  
 TA-100 ANTH 100 UG/PLATE

VI. INTERPRETATION OF RESULTS AND CONCLUSIONS

Compound: Sodium Ascorbate U.S.P., F.C.C., FDA 75-64, 000134-03-2.

A. Salmonella typhimurium

1. Plate Tests

The results of these tests were negative.

2. Nonactivation Suspension Tests

The results of these tests were negative. NA<sub>1</sub> dose with TA-1538 was repeated because of low mutant counts and NA<sub>1</sub> dose with TA-98 was repeated because of an increased mutant count. The repeat tests were negative.

3. Activation Suspension Tests

The results of these tests were negative. The LU<sub>1</sub> dose with TA-98 was repeated because of an increased mutant frequency. The repeat tests were negative. Slightly increased mutant frequencies were observed with TA-1538 using monkey lung tissue. Closer examination of the raw data indicated low population counts at these dose levels so that these increase were not considered significant.

B. Saccharomyces cerevisiae

1. Nonactivation Suspension Tests

The results of these tests were negative.

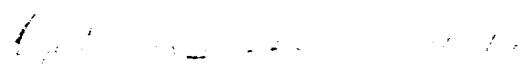
2. Activation Suspension Tests

The results of these tests were negative.

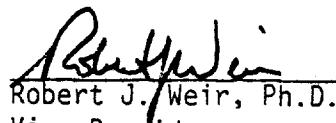
C. Conclusions

The test compound Sodium Ascorbate U.S.P., F.C.C., FDA 75-64, 000134-03-2, did not exhibit mutagenic activity in any of the assays employed in these studies.

Submitted by:

  
David J. Brusick, Ph.D. Date  
Director  
Department of Genetics

Reviewed by:

  
Robert J. Weir, Ph.D. Date  
Vice President



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## VII. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and cells are incubated in the overlay for 2-3 days, and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test.

- The small number of cell divisions permits potential mutagens to act on replicating DNA which is often more sensitive than non-replicating DNA.
- The combined incubation of the compound and the cells in the overlay permit constant exposure of the indicator cells for 2-3 days.

### A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs dose levels that are selected such that the highest dose will show slight toxicity (as determined by subjective criteria) and several doses ranging down 1 to 2 logs lower.

### B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. Factors which may modify dose response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced and the compound will not appear to be mutagenic.

### C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.



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#### D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and based primarily on a historical data base. Most data sets are evaluated using the following criteria:

##### 1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical which produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

##### 2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical which produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

##### 3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

##### 4. Reproducibility

If a chemical produces a response in a single test which cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.



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## VIII. EXPLANATION OF EVALUATION PROCEDURES FOR SUSPENSION ASSAYS

Data obtained from mutagenicity tests are evaluated on a test by test basis followed by an examination of the total response pattern using all the data. To facilitate this type of evaluation, we have prepared two separate formats in which data are processed. The first is the Compound Summary Backup Detail Sheet, which details the essential raw data from each experiment showing surviving population counts, total mutant or convertant counts, as well as, calculated mutation frequencies. This format permits close examination of each set of test data. The following considerations are part of any assessment.

### A. Surviving Population Counts

A certain level of chemically-induced toxicity is anticipated, but occasionally isolated tests or groups of tests show very low (<25%) survival compared to the tissue controls. Such isolated decreases may result from improper dilution procedures or defective growth media and decrease confidence in the calculated mutation frequencies especially if the total mutant counts appear unaffected. Data of this type are generally unacceptable and these experiments are routinely repeated at a lower dose level to reduce killing and increase confidence in the nature of the response.

### B. Total Mutant Counts

For nonmutagens, the mutant/surviving population ratio should be roughly equivalent for each test point in a given experiment. If the cell number drops in response to killing, the mutant number should decrease proportionately. A mutagenic chemical, however, will produce an altered mutant/surviving population ratio. Mutant numbers as well as calculated frequencies are compared to the negative control data. In certain instances, the mutant frequencies will increase with little or no change in the absolute number of mutants especially where the test chemical is toxic. Data of this type, although not necessarily aberrant, or even rare, must be viewed with special care to ensure that the increased frequencies were not the result of selective toxicity of the test chemical for the his<sup>-</sup> cells. This phenomenon, referred to as selection, can lead to erroneous conclusions. Thus we attempt to keep the surviving population of cells high and look for positive responses that show increases in both numbers of mutants and mutation frequencies. Again, occasional isolated fluctuations in mutant counts are found that can be attributed to improper pipetting or media contamination. These fluctuations are usually easy to identify by inspection of the other data points in the experiment which will be negative.



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### C. Dose Response Phenomena

Dose-related increases in mutants and mutation frequencies are the most convincing data to have in assessing mutagenic activity of chemicals. In some cases, however, dose-related increases are not observed for mutagens. This depends considerably on the dose levels selected. The figure on the following page illustrates how one might obtain various types of dose-related responses by a mutagen based solely on dose selection. It also emphasizes the need to keep dose levels within a relatively low range of toxicity so that data are consistently on the uphill side of the hypothetical curve.

### D. Control Tests

Positive and negative control tests are conducted with each experiment and consist of direct acting positive agents for nonactivation assays and chemicals that require metabolic transformation for activation assays. In nonactivation assays, the NAN control contain the test chemical solvent plus cells, but no chemical, and is used as a reference to assess the level of response obtained in the various tests. It is not possible at this time to put precise cut-off points where negative responses become positive responses. A statistical component for our computer program is under development and will be included when available. Positive controls are only used as relative reference points and to demonstrate that the system is functioning with known mutagens. In activation assays, three types of negative controls are run: (1) A solvent control minus the chemical and minus the activation system (A-C); (2) a control plus the positive control chemical minus the activation system (A+C); and (3) a control containing the activation system and the test chemical solvent (ALI or ALU). All three controls are used collectively to assess the level of response in the various activation tests. A chemical may appear positive when compared to an A-C control but not when compared to an A+T control. The value of each of the above controls with respect to their weight in evaluation is ALI or ALU > A-C > A+C.

The other data format is the Compound Frequency Summary Report sheet in which all the calculated frequencies obtained for a given compound are displayed in a table. This format permits an overview of all data. The points form a matrix of information that should present a consistent pattern. Nonmutagens should produce a matrix with data frequencies clustered around the negative control values. Occasional random high or low fluctuations are not uncommon and seldom indicate true genetic activity. Mutagenic chemicals should, on the other hand, produce a set of consistent responses that demonstrate a logical pattern. The patterns depend on the mutagenic specificity of the chemical but can be easily recognized in the Compound Frequency Summary Report format.

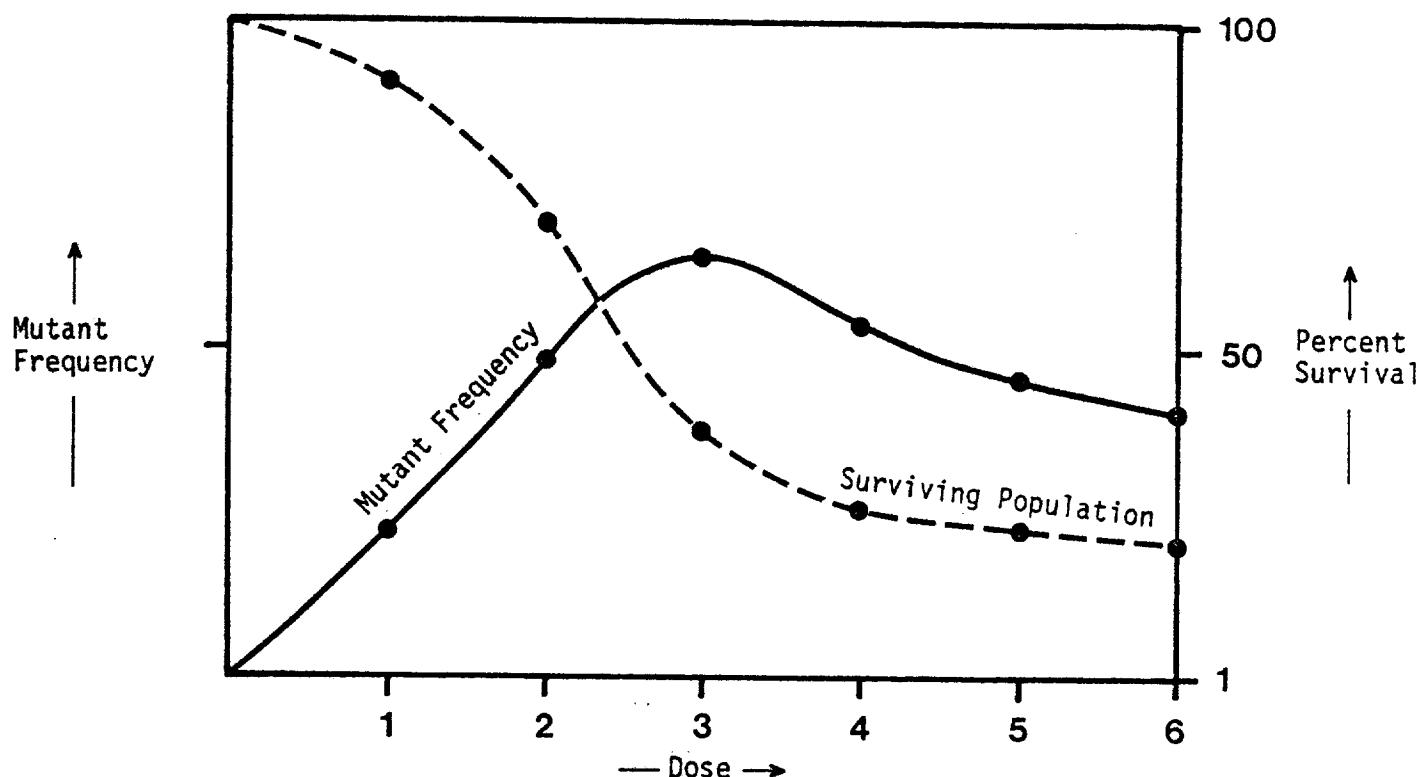
These mutagenicity assays are designed to optimize the probability of recognizing mutagens from nonmutagens and, in most cases, they work well. Occasionally, the data points are such that a definitive conclusion cannot be made without additional data.



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### HYPOTHETICAL MUTATION AND TOXICITY KINETICS



#### HYPOTHETICAL EXPERIMENT

- (1) Dose levels 1, 2 & 3 were used
- (2) Dose levels 2, 3 & 4 were used
- (3) Dose levels 3, 4 & 5 were used

#### OBSERVED DOSE RESPONSE

- A typical positive dose response set of data would be obtained.
- The intermediate dose level shows a higher mutation frequency than both the low dose and the high dose.
- Here an inverted dose response would be observed with the highest dose level showing the lowest response.

## STANDARD OPERATING PROCEDURES

To ensure an accurate and reliable mutagenicity testing program, LBI instituted the following procedures:

- The test compound was registered in a bound log book recording the date of receipt, complete client identification, physical description and LBI code number.
- Complete records of weights and dilutions associated with the testing of the submitted material were entered into a bound notebook.
- Raw data information was recorded on special printed forms that were dated and initialed by the individual performing the data collection at the time the observations were made. These forms were filed as permanent records.
- All animal tissue S-9 preparations used in the activation tests were taken from dated and pretested frozen lots identified by a unique number. The S-9 preparations were monitored for uniformity and the information recorded.



BIONETICS

APPENDIX

Tabulation of Data

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	22374-2104	PROJECT	02468	DATE
627205	DETECTOR	TA100	SPECIES	/	- 10/27/76
			POPU	MUT1	FREQ1
COMPOUND	TEST	ORG ID	CONCENTRATION	EP+6	EP-8
NAN		SOLVENT	0803	0535	66.63
NAP		EMS 0.066%	0548	4000	729.93
000134032	NA1	0003-1 PCT.	0713	0506	70.97
000134032	NA2	0015-2 PCT.	0784	0570	72.70
000134032	NA3	0075-3 PCT.	1407	0588	41.79

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 625801			CONTRACT 22374-2104	DETECTOR TA1535	SPECIES /	PROJECT 02468	DATE - 10/27/76
COMPOUND	TEST ID	ORG	CONCENTRATION	POPU	MUT1 EP+6	FREQ1 EP-8	CONTAM
NAN		SOLVENT		0257	0047	18.29	0
NAP		EMS 0.2%		0081	4000	4938.27	0
000134032	NA1	0003-1 PCT.		0230	0032	13.91	0
000134032	NA2	0015-2 PCT.		0221	0043	19.46	0
000134032	NA3	0075-3 PCT.		0274	0025	9.12	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	DETECTOR	TA1537	SPECIES	PROJECT	DATE
626501	22374-2104				02468	- 10/27/76
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUT1	FREQ1
				EP+6	EP+0	EP-B
NAN	SOLVENT			0221	0032	14.48
NAP	QM 13 UG/ML			0251	0240	95.62
000134032	NA1	0003-1 PCT.		0162	0022	13.58
000134032	NA2	0015-2 PCT.		0208	0027	12.98
000134032	NA3	0075-3 PCT.		0132	0039	29.55

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 626806 CONTRACT 22374-2104  
TEST 626806 DETECTOR TA1538 SPECIES /  
PROJECT 02468  
DATE - 10/27/76

COMPOUND	TEST ID	ORG	CONCENTRATION	POPUP	MUT1	FREQ1	CONTAM
NAN		SOLVENT		EP+6	EP+0	EP-6	
000134032	NA1	0003-1	PCT.	0365	0015	4.11	0
				0155	0010	6.45	0

REPORT EXP 33 LITTON HIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	TEST ID	CONTRACT	DETECTOR	TA1538	SPECIES	PROJECT	02468	DATE - 10/27/76
626402		22374-2104				/		
COMPOND	TEST ID	ORG	CONCENTRATION	POPUP	MUTL	FREQ1		
NAN		SOLVENT	EP+6	EP+6	EP+0	EP-8		
NAP		NF 667	UG/ML	0410	0590	143.90		
000134032	NA1	0003-1	PCT.	0384	0006	1.56		
000134032	NA2	0015-2	PCT.	0454	0012	2.64		
000134032	NA3	0075-3	PCT.	0600	0007	1.17		

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	TEST ID	SPECIES	PROJECT	DATE
628903	22374-2104	DETECTOR TA98	/	02468	10/27/76
COMPOUND	ORG ID	CONCENTRATION	POPU	MUT1	FREQ1
NAN	SOLVENT	EP+6	EP+0	EP-8	CONTAM
000134032	NA1	0003-1 PCT.	1008	0100	9.92
			0712	0104	14.61
					0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	22374-2104	DETECTOR TA98	SPECIES	PROJECT	02468	DATE	- 10/27/76
COMPOUND	TEST ID	ORG	CONCENTRATION	POPU	MUT1	FREQ1	EP-B	CONTAM
NAN		SOLVENT	EP+6	EP+0				
NAP		NF	667 UG/ML	0049	0402	13.86	0	
000134032	NA1	0003-1	PCT.	0120	0038	820.41	0	
000134032	NA2	0015-2	PCT.	0283	0043	31.67	0	
000134032	NA3	0075-3	PCT.	0300	0037	15.19	0	
						12.33	0	

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 628801			PROJECT 22374-2104	SPECIES			PROJECT 02468			DATE - 10/27/76		
COMPOUND	TEST ID	ORG	DETECTOR	CONCENTRATION	POPUP EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM		
NAN		SOLVENT			1118	0250	0109	22.36	9.75	0		
NAP		EMS 1.0 %			0294	0202	0112	68.71	38.10	0		
000134032	NA1	0005-0 PCT.			1216	0248	0297	20.39	24.42	0		
000134032	NA2	0025-1 PCT.			1160	0285	0125	24.57	10.78	0		
000134032	NA3	0125-2 PCT.			1270	0261	0146	20.55	11.50	0		

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 627805 CONTRACT 22374-2104  
SOLVENT DETECTOR TA100 SPECIES ICRFLU/MOUSE

PROJECT 02468 DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUT1 EP+6	FREQ1 EP+8	CONTAM
A+C		DMN 90 UM/ML	2342	0482	20.58	0	
A-C		SOLVENT	2274	0507	22.30	0	
ALI		TISSUE	2604	0636	24.42	0	
ALU		TISSUE	2104	0458	21.77	0	
ACP	LI	DMN 90 UM/ML	1400	0982	70.14	0	
ACP	LU	DMN 90 UM/ML	2506	0559	22.31	0	
000134032	ACT	LI1 0003-1 PCT.	2344	0545	23.25	0	
000134032	ACT	LI2 0015-2 PCT.	2028	0483	23.82	0	
000134032	ACT	LI3 0075-3 PCT.	2220	0554	24.95	0	
000134032	ACT	LU1 0003-1 PCT.	1962	0443	22.58	0	
000134032	ACT	LU2 0015-2 PCT.	1854	0438	23.62	0	
000134032	ACT	LU3 0075-3 PCT.	1952	0489	25.05	0	

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 625904 CONTRACT 22374-2104 DATE 10/15/75 SPECIES ICRLFLO/MOUSE PROJECT 02468 DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUT1	FREQ1	CONTAM
A+C		DMN 90 UM/ML	0488	0037	7.58	0	
A-C		SOLVENT	0539	0030	5.57	0	
ALI		TISSUE	0357	0023	6.44	0	
ALU		TISSUE	0363	0029	7.57	0	
ACP	LI	DMN 90 UM/ML	0353	0481	136.26	0	
ACP	LU	DMN 90 UM/ML	0337	0091	27.00	0	
000134032	ACT	LI1 0003-1 PCT.	0467	0037	7.92	0	
000134032	ACT	LI2 0015-2 PCT.	0356	0037	10.39	0	
000134032	ACT	LI3 0075-3 PCT.	0335	0049	14.63	0	
000134032	ACT	LU1 0003-1 PCT.	0494	0028	5.67	0	
000134032	ACT	LU2 0015-2 PCT.	0353	0020	5.67	0	
000134032	ACT	LU3 0075-3 PCT.	0444	0058	13.06	0	

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 629301 CONTRACT 22374-2104 DATE - 10/27/76  
DETECTOR TA1537 SPECIES ICRFLO/MOUSE PROJECT 02468

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUP	MUT1	FREQ1	
			AMQ 333 UG/ML	EP+6	EP+0	EP-8	CONTAM
A+C			AMQ 333 UG/ML	0595	0016	2.69	0
A-C			SOLVENT	0547	0032	5.85	0
ALI			TISSUE	0518	0036	6.95	2
ALU			TISSUE	0548	0020	3.65	0
ACP	LI	AMQ 333 UG/ML		0311	0444	142.77	0
ACP	LU	AMQ 333 UG/ML		0608	0013	2.14	0
000134032	ACT	L11 0003-1 PCT.		0648	0051	7.87	0
000134032	ACT	L12 0015-2 PCT.		0448	0043	9.60	0
000134032	ACT	L13 0075-3 PCT.		0586	0038	6.48	0
000134032	ACT	L11 0003-1 PCT.		0646	0047	7.28	0
000134032	ACT	LU2 0015-2 PCT.		0574	0047	8.19	0
000134032	ACT	LU3 0075-3 PCT.		0646	0051	7.89	0

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 626001 CONTRACT 22374-2104  
DETECTOR TA1538 SPECIES ICRFL0/MOUSE  
PROJECT 02468 DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUT1	FREQ1	CONTAM
				EP+6	EP+0	EP-8	
A+C		ANTH	67 UG/ML	0954	0277	29.04	0
A-C		SOLVENT		0862	0246	28.54	0
ALI		TISSUE		0523	0260	49.71	0
ALU		TISSUE		0924	0236	25.54	0
ACP	LI	ANTH	67 UG/ML	0449	0910	202.67	0
ACP	LU	ANTH	67 UG/ML	0842	0323	38.36	0
000134032	ACT	L11	0003-1 PCT.	2005	0271	13.52	0
000134032	ACT	L12	0015-2 PCT.	0748	0185	24.73	0
000134032	ACT	L13	0075-3 PCT.	0517	0176	34.04	0
000134032	ACT	LU1	0003-1 PCT.	0953	0155	16.26	0
000134032	ACT	LU2	0015-2 PCT.	0643	0154	23.95	0
000134032	ACT	LU3	0075-3 PCT.	0851	0186	21.86	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	22374-2104 DETECTOR TA98	SPECIES	PROJECT 02468 ICRFLO/MOUSE	DATE - 10/27/76
COMPOUND	TEST ID	ORG CONCENTRATION	POPU	MUT1	FREQ1
ALU	TISSUE		EP+6	EP+0	EP-8
000134032	ACT	L01 0005-0 PCT.	1225	0106	8.65
			0814	0133	16.34
					0

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 627206 CONTRACT 22374-2104 DETECTOR TA98 SPECIES ICRFLO/MOUSE

PROJECT 02468 DATE - 10/27/76

COMPOUND	TEST ID	ORG	CONCENTRATION	POPUP	MUT 1	FREQ1	CONTAM
				EP+6	EP+0	EP-8	
A+C		ANTH	67 UG/ML	0657	0031	4.72	0
A-C		SOLVENT		1003	0031	3.09	0
ALI		TISSUE		0714	0071	9.94	0
ALU		TISSUE		0702	0038	5.41	0
ACP	LI	ANTH	67 UG/ML	0603	0641	106.30	0
ACP	LU	ANTH	67 UG/ML	0770	0850	110.39	0
000134032	ACT	L11	0003-1 PCT.	0421	0041	9.74	0
000134032	ACT	L12	0015-2 PCT.	0289	0044	15.22	0
000134032	ACT	L13	0075-3 PCT.	0326	0064	19.63	0
000134032	ACT	L01	0003-1 PCT.	0264	0123	46.59	0
000134032	ACT	LU2	0015-2 PCT.	0439	0058	13.21	0
000134032	ACT	LU3	0075-3 PCT.	0468	0063	13.46	0

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 629202 CONTRACT 22374-2104 DETECTOR 000004 SPECIES ICRFLO/MOUSE PROJECT 02468  
DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUP EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
A+C		DMN 90 UM/ML	1474	0343	0120	23.27	8.14	0	
A-C		SOLVENT	1304	0341	0104	26.15	7.98	0	
• ALI		TISSUE	1244	0330	0108	26.53	8.68	0	
ALU		TISSUE	1194	0345	0103	28.89	8.63	0	
ACP	LI	DMN 90 UM/ML	0848	0574	0232	67.69	27.36	0	
ACP	LU	DMN 90 UM/ML	1111	0360	0127	32.40	11.43	0	
000134032	ACT	LI1 0005-0 PCT.	1116	0241	0127	21.59	11.38	0	
000134032	ACT	LI2 0025-1 PCT.	1128	0225	0094	19.95	8.33	0	
000134032	ACT	LI3 0125-2 PCT.	1280	0342	0094	26.72	7.34	0	
000134032	ACT	LU1 0005-0 PCT.	1130	0329	0134	29.12	11.86	0	
000134032	ACT	LU2 0025-1 PCT.	1098	0286	0094	26.05	8.56	0	
000134032	ACT	LU3 0125-2 PCT.	1012	0282	0099	27.87	9.78	0	

REPORT EXP33 LITTON RIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 627801 CONTRACT 22374-2104  
PROJECT 02468 DATE - 10/27/76  
DETECTOR TA100 SPECIES SPRDAW/RAT

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-B	CONTAM
A+C		DMN 90 UM/ML	2500	0518	20.72	0	
A-C		SOLVENT	2288	0591	25.83	0	
ALI		TISSUE	2172	0683	31.45	0	
ALU		TISSUE	2008	0543	27.04	0	
ACP	L1	DMN 90 UM/ML	1419	0876	61.73	0	
ACP	LU	DMN 90 UM/ML	2254	0636	28.22	0	
000134032	ACT	LJ1 0003-1 PCT.	2250	0719	31.96	0	
000134032	ACT	LJ2 0015-2 PCT.	2146	0860	40.07	0	
000134032	ACT	LJ3 0075-3 PCT.	2402	0860	35.80	0	
000134032	ACT	LU1 0003-1 PCT.	1062	0617	58.10	0	
000134032	ACT	LU2 0015-2 PCT.	1956	0640	32.72	0	
000134032	ACT	LU3 0075-3 PCT.	1968	0683	34.71	0	

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 626701 CONTRACT 22374-2104  
DETECTOR TA1535 SPECIES SPRDAW/RAT

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUP	MUT1	FREQ1	CONTAM
A+C		DMN 90 UM/ML	0909	0098	10.78	0	
A-C	SOLVENT		1063	0108	10.16	0	
ALI	TISSUE		0672	0091	13.54	0	
ALU	TISSUE		0823	0104	12.64	2	
ACP	LI	DMN 90 UM/ML	1272	3140	246.86	0	
ACP	LU	DMN 90 UM/ML	0681	0096	13.97	2	
000134032	ACT	LI1 0003-1 PCT.	0570	0093	16.32	2	
000134032	ACT	LI2 0015-2 PCT.	0814	0125	15.36	2	
000134032	ACT	LI3 0075-3 PCT.	0640	0083	12.97	2	
000134032	ACT	LU1 0003-1 PCT.	1411	0079	5.58	0	
000134032	ACT	LU2 0015-2 PCT.	1504	0067	4.45	2	
000134032	ACT	LU3 0075-3 PCT.	1297	0078	6.01	0	

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 629501 CONTRACT 22374-2104 PROJECT 02468  
DETECTOR TA1537 SPECIES SPRDAW/RAT DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUT1	FREQ1	CONTAM
				EP+6	EP+0	EP-8	
A+C		AMQ 333 UG/ML	0600	0025	4.17	0	
A-C		SOLVENT	0641	0017	2.65	0	
ALI		TISSUE	0669	0025	3.74	1	
ALU		TISSUE	0568	0012	2.11	0	
ACP	LI	AMQ 333 UG/ML	0200	0245	122.50	1	
ACP	LU	AMQ 333 UG/ML	0582	0008	1.37	2	
000134032	ACT	L11 0003-1 PCT.	0569	0017	2.99	0	
000134032	ACT	L12 0015-2 PCT.	0592	0016	2.70	0	
000134032	ACT	L13 0075-3 PCT.	0585	0017	2.91	0	
000134032	ACT	L01 0003-1 PCT.	0590	0012	2.03	1	
000134032	ACT	LU2 0015-2 PCT.	0574	0012	2.09	1	
000134032	ACT	LU3 0075-3 PCT.	0618	0012	1.94	1	

REPORT EXP33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

COMPOUND	TEST	ORG ID	CONCENTRATION	PROJECT 02468		DATE - 10/27/76		
				POP/EP+6	MUT/EP+0			
A+C	ANTH	67	UG/ML	0427	0021	4.92	0	
A-C	SOLVENT			0486	0020	4.12	0	
ALI	TISSUE			0271	0050	16.45	0	
ALU	TISSUE			0374	0028	7.49	0	
ACP	LI	ANTH	67	UG/ML	0269	0588	218.59	0
ACP	LU	ANTH	67	UG/ML	0250	0683	273.20	0
000134032	ACT	L11	0003-1	PCT.	0183	0027	14.75	0
000134032	ACT	L12	0015-2	PCT.	0166	0028	16.87	0
000134032	ACT	L13	0075-3	PCT.	0344	0024	6.98	0
000134032	ACT	L01	0003-1	PCT.	0186	0031	16.67	0
000134032	ACT	L02	0015-2	PCT.	0252	0033	13.10	0
000134032	ACT	L03	0075-3	PCT.	0292	0024	8.22	0

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

COMPOUND	TEST	ORG ID	CONCENTRATION	PROJECT 02468		DATE - 10/27/76
				SPCIES	SPRDW/RAT	
A+C	ANTH	67	UG/ML	1712	0212	15.89
A-C	SOLVENT			2147	0261	12.44
ALI	TISSUE			2557	0297	11.62
ALU	TISSUE			1650	0331	20.06
ACP	LI	ANTH	67 UG/ML	1116	0938	84.05
ACP	LU	ANTH	67 UG/ML	1304	0324	24.05
000134032	ACT	L11	0003-1 PCT.	1015	0224	22.07
000134032	ACT	L12	0015-2 PCT.	0885	0231	26.10
000134032	ACT	L13	0075-3 PCT.	0940	0229	24.36
000134032	ACT	LU1	0003-1 PCT.	1308	0357	27.29
000134032	ACT	LU2	0015-2 PCT.	1468	0280	19.07
000134032	ACT	LU3	0075-3 PCT.	1394	0279	20.01

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 629502 CONTRACT 22374-2104 DETECTOR 000004 PROJECT 02468  
SPECIES SPRDAW/RAT DATE - 10/27/76

COMPOUND	TEST ID	ORG	CONCENTRATION	POPU	MUT1	MUT2	FREQ1	FREQ2	
				EP+4	EP+1	EP+1	EP-5	EP-5	CONTAM
A+C		DMN	90 UM/ML	0886	0381	0191	43.00	21.56	0
A-C		SOLVENT		0761	0390	0155	51.25	20.37	0
ALI		TISSUE		0708	0296	0187	41.81	26.41	0
ALU		TISSUE		0726	0284	0153	39.12	21.07	0
ACP	LI	DMN	90 UM/ML	0567	0453	0343	79.89	60.49	0
ACP	LU	DMN	90 UM/ML	0826	0338	0186	40.92	22.52	0
000134032	ACT	L11	0005-0 PCT.	0837	0321	0124	38.35	14.81	0
000134032	ACT	L12	0025-1 PCT.	0750	0324	0133	43.20	17.73	0
000134032	ACT	L13	0125-2 PCT.	0611	0300	0134	49.10	21.93	0
000134032	ACT	LU1	0005-0 PCT.	0754	0298	0103	39.52	13.66	0
000134032	ACT	LU2	0025-1 PCT.	0712	0293	0101	41.15	14.19	0
000134032	ACT	LU3	0125-2 PCT.	0573	0279	0091	48.69	15.88	0

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 627901 CONTRACT 22374-2104 DETECTOR TA100 SPECIES RHECUS/MONKEY PROJECT 02468 DATE - 10/27/76

COMPOUND	TEST ID	ORG	CONCENTRATION	POPU	MUT1	FREQ1	CONTAM
		A+C	DMN 90 UM/ML	EP+6	EP+0	EP~8	
A-C	SOLVENT			1842	0476	25.84	0
ALI	TISSUE			2708	0814	30.06	0
ALU	TISSUE			2442	0699	28.62	0
ACP	LI	DMN 90 UM/ML		1104	0713	60.22	0
ACP	LU	DMN 90 UM/ML		2430	0752	30.95	0
000134032	ACT	L11	0003-1 PCT.	2344	0707	30.16	0
000134032	ACT	L12	0015-2 PCT.	1932	0619	32.04	0
000134032	ACT	L13	0075-3 PCT.	2454	0650	26.49	0
000134032	ACT	L01	0003-1 PCT.	2148	0664	30.91	0
000134032	ACT	L02	0015-2 PCT.	2312	0618	26.73	0
000134032	ACT	L03	0075-3 PCT.	2570	0748	29.11	0

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104  
EXPERIMENT 62R001 DETECTOR TA1535 SPECIES RHECUS/MONKEY

PROJECT 02468  
DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUP EP+6	MUT1 EP+0	FREQ1 EP-B	CONTAM
A+C		DMN 90 UM/ML	2201	0164		7.45	0
A-C		SOLVENT	2313	0223		9.64	0
ALI		TISSUE	2757	0190		6.89	0
ALU		TISSUE	2683	0203		7.57	0
ACP	LI	DMN 90 UM/ML	1754	1020		58.15	0
ACP	LU	DMN 90 UM/ML	2777	0245		8.82	0
000134032	ACT	L11 0005-0 PCT.	2400	0185		7.71	0
000134032	ACT	L12 0025-1 PCT.	2493	0240		9.63	0
000134032	ACT	L13 0125-2 PCT.	2219	0169		7.62	0
000134032	ACT	LU1 0005-0 PCT.	2816	0195		6.92	0
000134032	ACT	LU2 0025-1 PCT.	2469	0196		7.94	0
000134032	ACT	LU3 0125-2 PCT.	2870	0196		6.83	0

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 629701 CONTRACT 22374~2104 DETECTOR TA1537 PROJECT 02468  
DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUP	MUT1	FREQ1	CONTAM
A+C		AMQ 333	06/ML	2774	0327	11.79	2
A-C		SOLVENT		1818	0145	7.98	2
ALI	TISSUE			0981	0181	18.45	2
ALU	TISSUE			0996	0221	22.19	2
ACP	LI	AMQ 333	06/ML	2119	0065	3.07	2
ACP	LU	AMQ 333	06/ML	2400	0315	13.13	2
000134032	ACT	L11	0003-1 PCT.	0841	0165	19.62	0
000134032	ACT	L12	0015-2 PCT.	0787	0161	20.46	0
000134032	ACT	L13	0075-3 PCT.	0896	0177	19.75	0
000134032	ACT	L01	0003-1 PCT.	1087	0266	24.47	0
000134032	ACT	L02	0015-2 PCT.	0739	0337	45.60	0
000134032	ACT	L03	0075-3 PCT.	1146	0269	23.47	0

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 627104 CONTRACT 22374-2104 DATE 10/27/76  
DETECTOR TA1538 SPECIES RHESUS/MONKEY PROJECT 02468

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUP	MUT1	FREQ1	CONTAM
				EP+6	EP+0	EP-8	
A+C		ANTH 67	UG/ML	0544	0018	3.31	0
A-C		SOLVENT		0306	0019	6.21	0
ALI		TISSUE		0418	0045	10.77	0
ALU		TISSUE		0554	0021	3.79	0
ACP	LI	ANTH 67	UG/ML	0334	1940	580.84	0
ACP	LU	ANTH 67	UG/ML	0674	0026	3.86	0
000134032	ACT	L11	0003-1 PCT.	0194	0023	11.86	0
000134032	ACT	L12	0015-2 PCT.	0198	0009	4.55	0
000134032	ACT	L13	0075-3 PCT.	0194	0020	10.31	0
000134032	ACT	LU1	0003-1 PCT.	0110	0019	17.27	0
000134032	ACT	LU2	0015-2 PCT.	0080	0012	15.00	0
000134032	ACT	LU3	0075-3 PCT.	0180	0009	5.00	0

REPORT EXH33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 627401 CONTRACT 22374-2104  
DETECTOR TA98

PROJECT 02468  
SPECIES RHECUS/MONKEY

DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUT1	FREQ1	CONTAM
				EP+6	EP+0	EP-8	
A+C		ANTH 67	UG/ML	1559	0412	26.43	1
A-C		SOLVENT		1803	0489	27.12	0
ALI		TISSUE		0916	0518	56.55	0
ALU		TISSUE		0753	0463	61.49	1
ACP	L1	ANTH 67	UG/ML	1088	0963	88.51	1
ACP	LU	ANTH 67	UG/ML	0953	0409	42.92	0
000134032	ACT	L11	0003-1 PCT.	0982	0403	41.04	1
000134032	ACT	L12	0015-2 PCT.	1362	0374	27.46	0
000134032	ACT	L13	0075-3 PCT.	1235	0382	30.93	0
000134032	ACT	LU1	0003-1 PCT.	1185	0470	39.66	1
000134032	ACT	LU2	0015-2 PCT.	1069	0443	41.44	0
000134032	ACT	LU3	0075-3 PCT.	1229	0429	34.91	0

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

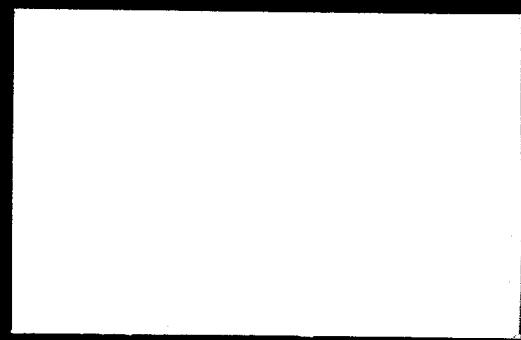
EXPERIMENT 629503 CONTRACT 22374-21<sup>04</sup> DETECTOR 000004 SPECIES RHECUS/MONKEY PROJECT 02468 DATE - 10/27/76

COMPOUND	TEST ID	ORG	CONCENTRATION	POPUP EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
A+C	DMN 90 UM/ML		0787	0104	0059	13.21	7.50	0	
A-C	SOLVENT		0624	0101	0047	16.19	7.53	0	
ALI	TISSUE		0751	0118	0058	15.71	7.72	0	
ALU	TISSUE		0734	0064	0042	8.72	5.72	0	
ACP	L1	DMN 90 UM/ML	0721	0486	0177	67.41	24.55	0	
ACP	LU	DMN 90 UM/ML	0735	0095	0045	12.93	6.12	0	
000134032	ACT	L11 0005-0 PCT.	0798	0118	0053	14.79	6.64	0	
000134032	ACT	L12 0025-1 PCT.	0672	0113	0049	16.82	7.29	0	
000134032	ACT	L13 0125-2 PCT.	0736	0102	0044	13.86	5.98	0	
000134032	ACT	L01 0005-0 PCT.	0734	0089	0039	12.13	5.31	0	
000134032	ACT	LU2 0025-1 PCT.	0728	0086	0045	11.81	6.18	0	
000134032	ACT	LU3 0125-2 PCT.	0790	0090	0061	11.39	7.72	0	



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Kensington, Maryland  
20795

BRUSICK

MUTAGENICITY EVALUATION  
OF  
SODIUM ASCORBATE U.S.P., F.C.C.  
FDA 75-64

FINAL REPORT

SUBMITTED TO

FOOD AND DRUG ADMINISTRATION  
DEPARTMENT OF HEALTH, EDUCATION AND WELFARE  
5600 FISHERS LANE  
ROCKVILLE, MARYLAND

SUBMITTED BY

LITTON BIONETICS, INC.  
5516 NICHOLSON LANE  
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 2672

OCTOBER 29, 1976



BIONETICS

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EVALUATION SUMMARY

The test compound Sodium Ascorbate U.S.P., F.C.C., FDA 75-64, 000134-03-2, did not exhibit mutagenic activity in any of the assays employed in these studies.



LITTON BIONETICS

DATE: October 29, 1976

SPONSOR: U.S. Food and Drug Administration

SUBJECT: Evaluation of Test Compound Sodium Ascorbate U.S.P., F.C.C., FDA 75-64

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: September 3, 1976
2. Description: white powder

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain: Saccharomyces cerevisiae, strain D4

Bacteria Strains: Salmonella typhimurium, strains TA-1535  
TA-1537  
TA-1538  
TA-98  
TA-100

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

<u>Component</u>	<u>Final Concentration/ml</u>
1. TPN (sodium salt)	4 $\mu$ moles
2. Glucose-6-Phosphate	5 $\mu$ moles
3. Sodium Phosphate (dibasic) pH 7.4	100 $\mu$ moles
4. $MgCl_2$	8 $\mu$ moles
5. KCl	33 $\mu$ moles
6. Homogenate fraction equivalent to 25 mg of wet tissue.	

D. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from tissues of the following mammalian species: Mouse - ICR random bred adult males; rat - Sprague-Dawley adult males; and monkey - Macaca mulatta adult males.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1  
POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

Assay	Chemical <sup>a</sup>	Solvent	Probable Mutagenic Specificity
Nonactivation	Methylnitrosoguanidine	Water or saline	BPS <sup>b</sup>
	Ethylmethanesulfonate	Water or saline	BPS <sup>b</sup>
	2-Nitrofluorene	Dimethylsulfoxide <sup>c</sup>	FS <sup>b</sup>
	Quinacrine mustard	Water or saline	FS
Activation	Dimethylnitrosamine	Water or saline	BPS <sup>b</sup>
	2-Acetylaminofluorene	Dimethylsulfoxide <sup>c</sup>	FS <sup>b</sup>
	8-Aminoquinoline	Dimethylsulfoxide <sup>c</sup>	FS <sup>b</sup>
	2-Aminoanthracene	Dimethylsulfoxide <sup>c</sup>	BPS <sup>b</sup>

<sup>a</sup> Concentrations given in the Results Section

<sup>b</sup> BPS = base-pair substitution; FS = frameshift

<sup>c</sup> Previously shown to be non-mutagenic

III. METHODS

A. Toxicity

The solubility, toxicity and doses for the test chemical were determined prior to screening.

The test chemical was tested for toxicity against specific indicator strains over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival concentrations and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for the chemical with a given strain, then a maximum dose of 5% (w/v) was used.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.



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B. Plate Tests (Overlay Method)

Approximately  $10^8$  cells from an overnight culture of each indicator strain were added to test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For nonactivation tests, the three dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests 0.5 ml of a 9,000 x g tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. Three dose levels of the test chemical were added to the appropriate tubes, which were then mixed and the contents poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. The plates were incubated for 48 to 72 hours at 37°C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using positive compounds that are active directly and those that require metabolic activation were run with each assay.

C. Suspension Tests

1. Nonactivation

Bacteria and yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of  $1 \times 10^{10}$  cells/ml and  $5 \times 10^9$  cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic, 24-well tissue culture plates (Linbro). Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.5 ml. The solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a  $10^{-1}$  dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the nonactivation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for nonactivation tests.



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D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (except monkeys) sufficient to provide the necessary quantities of tissues were killed by cranial blow, decapitated and bled. Monkey tissues were obtained from freshly killed and bled male rhesus monkeys. Organs were immediately dissected from the animals using aseptic techniques and placed in ice-cold 0.15 M KCl. Upon collection of the desired quantity of organs, they were washed twice with fresh KCl and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies. Protein and P-448 determinations were made for each lot of homogenate.

E. Data Recording and Reporting

1. Suspension assays

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. The data were then processed and printed from a computer program. All raw data sheets are dated and signed by the responsible technician.

2. Plate test assays

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were entered into a computer program designed to print out all data by test. The data are presented as revertants per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points.

IV. RESULTS SECTION

A. Solubility Properties of the Test Compound

1. Name or code designation of the test compound: 000134-03-2
2. Test solvent: Saline
3. Solubility of the test compound under treatment conditions:  
Soluble
4. Additional comments: White powder

B. Toxicity and Dosage Determinations for the Test Compound

1. Test date for toxicity determination: September 8, 1976
2. The 50% survival level was determined for bacteria and yeast indicator organisms by conducting survival curves with the test compound at the following concentrations:

Percent Concentration (w/v or v/v)

5.0  
0.5  
0.05  
0.005  
0.0005

3. Concentrations of the test compound used in the mutagenicity tests:

<u>Test Doses</u>	<u>Percent Concentration</u>	
	Bacteria	Yeast
1/4 50% Survival	0.075	1.25
1/2 50% Survival	0.150	2.50
50% Survival	0.300	5.00

C. Suspension Assay Results

The suspension test results for the test compound are summarized in the following six tables. The values presented in these tables are the calculated mutation frequencies for each control and experimental test point. The first table of the suspension set presents the results for the nonactivation assays, and the second through the fourth table of the suspension set presents the results for the activation assays. The fifth table shows the results of the nonactivation plate test and the sixth table shows the results of the activation plate test. A listing of computer codes and abbreviations is included for reference. Tabulation of all raw data is provided in the Appendix.



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DATA TABLE TERMS AND ABBREVIATIONS (continued)

<u>ABBREVIATION OR TERM</u>	<u>DEFINITION OR EXPLANATION</u>
AAF	2-Acetylaminofluorene
DMSO	Dimethylsulfoxide
DMN	Dimethylnitrosamine
EMS	Ethylmethanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
ANTH	2-Amino Anthracene
AMQ	8-Amino Quinoline
SPECIES	Animal Strains
SPRDW	Sprague Dawley Rats
ICRFLO	Flow ICR Random Bred Mice
RHESUS	Rhesus Monkey ( <u>Macaca mulatta</u> )
MIXEDB	Dog, Mixed Breed
NEWZEA	New Zealand White Rabbit
UG	Microgram
UM	Micromole
ADE	Adenine
TRY	Tryptophan



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LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 10/27/76

SPECIES / NONACTIVATION COMPOUND 000134032

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 ADE EX-5
NAN		66.63	10.29	14.48	1.21	4.11	13.86	9.92	22.36
NAP		729.93	4938.27	95.62	143.90	020.41		68.71	38.10
NA1		70.97	13.91	13.58	1.56	6.45	31.67	14.61	20.39
NA2		72.70	19.46	12.98	2.64		15.19		24.57
NA3		41.79	9.12	29.55	1.17		12.33		10.78
								20.55	11.50
									TEST DATA

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 10/27/76

SPECIES ICRFLO/HOUSE COMPOUND 000134032

TEST	ORG	TAI00 HIS EX-8	TAI535 HIS EX-8	TAI537 HIS EX-8	TAI538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	0000D4 ADE EX-5	0000D4 TRY EX-5
ACT	A+C	20.58	7.58	2.69	29.04	4.72		23.27		8.14
ACT	A-C	22.30	5.57	5.85	28.54	3.09		26.15		7.98
ACT	AL1	24.42	6.44	6.95	49.71	9.94		26.53		8.68
ACT	ALU	21.17	7.57	3.65	25.54	5.41	8.65	28.89		8.63
ACT	PLI	70.14	136.26	142.17	202.67	106.30		67.69		27.36
ACT	PLU	22.31	27.00	2.14	38.36	110.39		32.40		11.43
ACT	L11	23.25	7.92	7.87	13.52	9.74		21.59		11.38
ACT	L12	23.82	10.39	9.60	24.73	15.22		19.95		8.33
ACT	L13	24.95	14.63	6.48	34.04	19.63		26.72		7.34
ACT	L01	22.58	5.67	7.28	16.26	46.59	16.34	29.12		11.86
ACT	L02	23.62	5.67	8.19	23.95	13.21		26.05		8.56
ACT	L03	25.05	13.06	7.89	21.86	13.46		27.87		9.78

NEGATIVE CONTROL

POSITIVE CONTROL

TEST DATA

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 10/27/76

SPECIES SPHDW/RAF

COMPOUND 000134032

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-H	TA98 HIS EX-H	0000D4 AOE EX-5	0000D4 TRY EX-5	COMPOUND 000134032
ACT	A+C	20.72	10.78	4.17	4.92	15.09	43.00	21.56
ACT	A-C	25.83	10.16	2.65	4.12	12.44	51.25	20.37
ACT	ALI	31.45	13.54	3.74	18.45	11.62	41.81	26.41
ACT	ALU	27.04	12.64	2.11	7.49	20.06	39.12	21.07
ACT	PLI	61.73	246.86	122.50	218.59	84.05	79.89	60.49
ACT	PLU	28.22	13.97	1.37	273.20	24.85	40.92	22.52
ACT	LII	31.96	16.32	2.99	14.75	22.07	38.35	14.81
ACT	LII2	40.07	15.36	2.70	16.87	26.10	43.20	17.73
ACT	LII3	35.80	12.97	2.91	6.98	24.36	49.10	21.93
ACT	LUI	58.10	5.58	2.03	16.67	27.29	39.52	13.66
ACT	LU2	32.72	4.45	2.09	13.10	19.07	41.15	14.19
ACT	LU3	34.71	6.01	1.94	8.22	20.01	48.69	15.88

LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM  
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 10/27/76

SPECIES RHECUS/MONKEY      COMPOUND 000134032

TEST	ORG	TA100 HIS Ex-8	TA1535 HIS Ex-8	TA1537 HIS Ex-8	TA1538 HIS Ex-8	TA98 HIS Ex-8	0000D4 ADE Ex-5	0000D4 TRY Ex-5
ACT	A+C	26.73	7.45	11.79	3.31	26.43	13.21	7.50
ACT	A-C	25.84	9.64	1.98	6.21	27.12	16.19	7.53
ACT	ALI	30.06	6.89	18.45	10.77	56.55	15.71	7.72
ACT	ALU	28.62	7.57	22.19	3.79	61.49	8.72	5.72
ACT	PLI	60.22	58.15	3.07	580.84	88.51	67.41	24.55
ACT	PLU	30.95	8.82	13.13	3.86	42.92	12.93	6.12
ACT	LII	30.16	7.71	19.62	11.86	41.04	14.79	6.64
ACT	L12	32.04	9.63	20.46	4.55	27.46	16.82	7.29
ACT	L13	26.49	7.62	19.75	10.31	30.93	13.86	5.98
ACT	L01	30.91	6.92	24.47	17.27	39.66	12.13	5.31
ACT	L02	26.73	7.94	45.60	15.00	41.44	11.81	6.18
ACT	L03	29.11	6.83	23.47	5.00	34.91	11.39	7.72

## SUMMARY\_OF\_TEST\_RESULTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 000134032  
 H. TEST DATE: OCT. 12, 1976

TEST	SPECIES	ISSUE	PLATE_IESIS		PLATE_BIANS		PLATE_F-B		PLATE	
			IA-1535	IA-1537	IA-1539	IA-1530	IA-98	IA-100	IA-98	IA-100
1. NON-ACTIVATION	SOLVENT CONTROL*		---	31	23	31	19	18	22	21
	POSITIVE CONTROL**		---	>1000	>1000	895	>1000	>1000	>1000	201
TEST	0.30000 %		---	34	22	26	9	26	40	248
	0.15000 %		---	23	21	40	30	16	12	22
	0.07500 %		---	28	36	16	16	19	21	291
										196
2. ACTIVATION	SOLVENT CONTROL*		MOUSE	LIVER	25	40	20	12	22	24
			RAT	LIVER	20	20	14	11	32	19
			MONKEY	LIVER	16	41	12	6	22	89
			MOUSE	LIVER	202	154	303	516	>1000	77
TEST	0.30000 %		RAT	LIVER	94	91	>1000	127	462	500
	0.15000 %		MONKEY	LIVER	513	375	40	119	>1000	129
	0.07500 %		MOUSE	LIVER	21	26	25	15	21	123
			MOUSE	LIVER	27	35	26	17	19	100
			MOUSE	LIVER	39	54	15	20	20	154
			RAT	LIVER	15	19	12	14	17	181
			RAT	LIVER	18	18	6	19	13	285
			RAT	LIVER	16	23	13	8	30	167
			MONKEY	LIVER	37	30	9	13	24	150
			MONKEY	LIVER	31	40	13	10	21	160
			MONKEY	LIVER	25	36	14	8	13	151
										156

\* NON-ACTIVATION ASSAYS CONSIST OF THE CELLS PLUS THE TEST COMPOUND VEHICLE (SOLVENT). FOR ACTIVATION ASSAYS, THE OVERLAY CONTAINS THE ACTIVATION SYSTEM PLUS THE TEST COMPOUND VEHICLE.

\*\* TA-1535 MNNG 2 ug/PLATE TA-1535 ANTH 100 ug/PLATE  
 TA-1537 NM 20 ug/PLATE TA-1537 AMQ 100 ug/PLATE  
 TA-1538 NF 100 ug/PLATE TA-1538 AAF 100 ug/PLATE  
 TA-98 NF 100 ug/PLATE TA-98 AAF 100 ug/PLATE  
 TA-100 MNNG 2 ug/PLATE TA-100 ANTH 100 ug/PLATE  
 NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS(UL) OR MICROGRAMS(UG) PER PLATE.

VI. INTERPRETATION OF RESULTS AND CONCLUSIONS

Compound: Sodium Ascorbate U.S.P., F.C.C., FDA 75-64, 000134-03-2.

A. Salmonella typhimurium

1. Plate Tests

The results of these tests were negative.

2. Nonactivation Suspension Tests

The results of these tests were negative. NA<sub>1</sub> dose with TA-1538 was repeated because of low mutant counts and NA<sub>1</sub> dose with TA-98 was repeated because of an increased mutant count. The repeat tests were negative.

3. Activation Suspension Tests

The results of these tests were negative. The LU<sub>1</sub> dose with TA-98 was repeated because of an increased mutant frequency. The repeat tests were negative. Slightly increased mutant frequencies were observed with TA-1538 using monkey lung tissue. Closer examination of the raw data indicated low population counts at these dose levels so that these increase were not considered significant.

B. Saccharomyces cerevisiae

1. Nonactivation Suspension Tests

The results of these tests were negative.

2. Activation Suspension Tests

The results of these tests were negative.

C. Conclusions

The test compound Sodium Ascorbate U.S.P., F.C.C., FDA 75-64, 000134-03-2, did not exhibit mutagenic activity in any of the assays employed in these studies.

Submitted by:

/ \_\_\_\_\_  
David J. Brusick, Ph.D. Date  
Director  
Department of Genetics

Reviewed by:

*Robert J. Weir* 10/28/73  
Robert J. Weir, Ph.D. Date  
Vice President



BIONETICS

## VII. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and cells are incubated in the overlay for 2-3 days, and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test.

- The small number of cell divisions permits potential mutagens to act on replicating DNA which is often more sensitive than non-replicating DNA.
- The combined incubation of the compound and the cells in the overlay permit constant exposure of the indicator cells for 2-3 days.

### A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs dose levels that are selected such that the highest dose will show slight toxicity (as determined by subjective criteria) and several doses ranging down 1 to 2 logs lower.

### B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. Factors which may modify dose response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced and the compound will not appear to be mutagenic.

### C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

#### D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and based primarily on a historical data base. Most data sets are evaluated using the following criteria:

##### 1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical which produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

##### 2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical which produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

##### 3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

##### 4. Reproducibility

If a chemical produces a response in a single test which cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.



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## VIII. EXPLANATION OF EVALUATION PROCEDURES FOR SUSPENSION ASSAYS

Data obtained from mutagenicity tests are evaluated on a test by test basis followed by an examination of the total response pattern using all the data. To facilitate this type of evaluation, we have prepared two separate formats in which data are processed. The first is the Compound Summary Backup Detail Sheet, which details the essential raw data from each experiment showing surviving population counts, total mutant or convertant counts, as well as, calculated mutation frequencies. This format permits close examination of each set of test data. The following considerations are part of any assessment.

### A. Surviving Population Counts

A certain level of chemically-induced toxicity is anticipated, but occasionally isolated tests or groups of tests show very low (<25%) survival compared to the tissue controls. Such isolated decreases may result from improper dilution procedures or defective growth media and decrease confidence in the calculated mutation frequencies especially if the total mutant counts appear unaffected. Data of this type are generally unacceptable and these experiments are routinely repeated at a lower dose level to reduce killing and increase confidence in the nature of the response.

### B. Total Mutant Counts

For nonmutagens, the mutant/surviving population ratio should be roughly equivalent for each test point in a given experiment. If the cell number drops in response to killing, the mutant number should decrease proportionately. A mutagenic chemical, however, will produce an altered mutant/surviving population ratio. Mutant numbers as well as calculated frequencies are compared to the negative control data. In certain instances, the mutant frequencies will increase with little or no change in the absolute number of mutants especially where the test chemical is toxic. Data of this type, although not necessarily aberrant, or even rare, must be viewed with special care to ensure that the increased frequencies were not the result of selective toxicity of the test chemical for the his<sup>-</sup> cells. This phenomenon, referred to as selection, can lead to erroneous conclusions. Thus we attempt to keep the surviving population of cells high and look for positive responses that show increases in both numbers of mutants and mutation frequencies. Again, occasional isolated fluctuations in mutant counts are found that can be attributed to improper pipetting or media contamination. These fluctuations are usually easy to identify by inspection of the other data points in the experiment which will be negative.



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### C. Dose Response Phenomena

Dose-related increases in mutants and mutation frequencies are the most convincing data to have in assessing mutagenic activity of chemicals. In some cases, however, dose-related increases are not observed for mutagens. This depends considerably on the dose levels selected. The figure on the following page illustrates how one might obtain various types of dose-related responses by a mutagen based solely on dose selection. It also emphasizes the need to keep dose levels within a relatively low range of toxicity so that data are consistently on the uphill side of the hypothetical curve.

### D. Control Tests

Positive and negative control tests are conducted with each experiment and consist of direct acting positive agents for nonactivation assays and chemicals that require metabolic transformation for activation assays. In nonactivation assays, the NAN control contain the test chemical solvent plus cells, but no chemical, and is used as a reference to assess the level of response obtained in the various tests. It is not possible at this time to put precise cut-off points where negative responses become positive responses. A statistical component for our computer program is under development and will be included when available. Positive controls are only used as relative reference points and to demonstrate that the system is functioning with known mutagens. In activation assays, three types of negative controls are run: (1) A solvent control minus the chemical and minus the activation system (A-C); (2) a control plus the positive control chemical minus the activation system (A+C); and (3) a control containing the activation system and the test chemical solvent (ALI or ALU). All three controls are used collectively to assess the level of response in the various activation tests. A chemical may appear positive when compared to an A-C control but not when compared to an A+T control. The value of each of the above controls with respect to their weight in evaluation is ALI or ALU > A-C > A+C.

The other data format is the Compound Frequency Summary Report sheet in which all the calculated frequencies obtained for a given compound are displayed in a table. This format permits an overview of all data. The points form a matrix of information that should present a consistent pattern. Nonmutagens should produce a matrix with data frequencies clustered around the negative control values. Occasional random high or low fluctuations are not uncommon and seldom indicate true genetic activity. Mutagenic chemicals should, on the other hand, produce a set of consistent responses that demonstrate a logical pattern. The patterns depend on the mutagenic specificity of the chemical but can be easily recognized in the Compound Frequency Summary Report format.

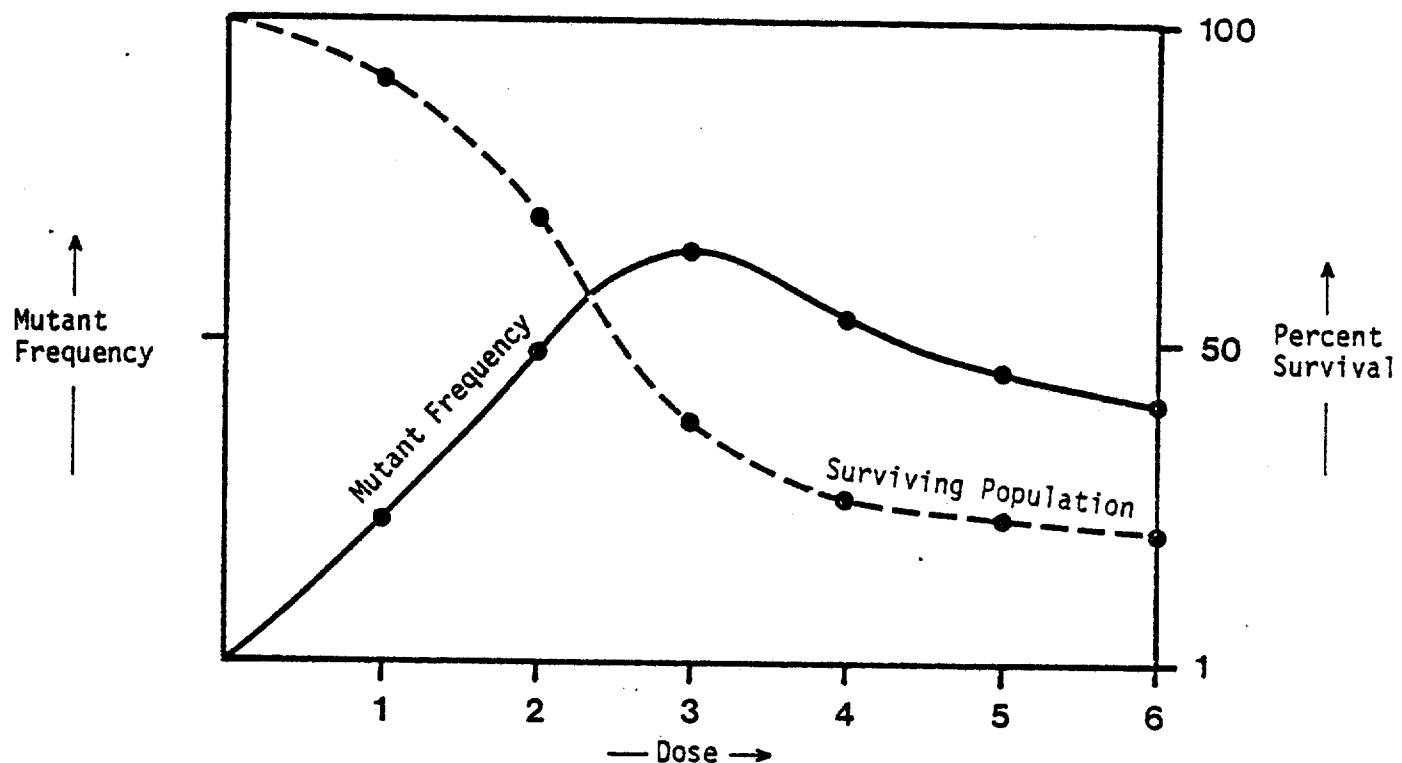
These mutagenicity assays are designed to optimize the probability of recognizing mutagens from nonmutagens and, in most cases, they work well. Occasionally, the data points are such that a definitive conclusion cannot be made without additional data.



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### HYPOTHETICAL MUTATION AND TOXICITY KINETICS



#### HYPOTHETICAL EXPERIMENT

- (1) Dose levels 1, 2 & 3 were used
- (2) Dose levels 2, 3 & 4 were used
- (3) Dose levels 3, 4 & 5 were used

#### OBSERVED DOSE RESPONSE

- A typical positive dose response set of data would be obtained.
- The intermediate dose level shows a higher mutation frequency than both the low dose and the high dose.
- Here an inverted dose response would be observed with the highest dose level showing the lowest response.

### STANDARD OPERATING PROCEDURES

To ensure an accurate and reliable mutagenicity testing program, LBI instituted the following procedures:

- The test compound was registered in a bound log book recording the date of receipt, complete client identification, physical description and LBI code number.
- Complete records of weights and dilutions associated with the testing of the submitted material were entered into a bound notebook.
- Raw data information was recorded on special printed forms that were dated and initialed by the individual performing the data collection at the time the observations were made. These forms were filed as permanent records.
- All animal tissue S-9 preparations used in the activation tests were taken from dated and pretested frozen lots identified by a unique number. The S-9 preparations were monitored for uniformity and the information recorded.



BIONETICS

APPENDIX  
Tabulation of Data



Litton BIONETICS

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 627205			CONTRACT 22374-2104			PROJECT 02460			DATE - 10/27/76	
COMPOUND	TEST ID	ORG ID	CONCENTRATION	SPECIES	/	MUT1	MUT2	FREQ1	FREQ2	CONTAM
NAN			SOLVENT	0803	0535			66.63		0
NAP			FMS 0.066%	0548	4000			729.93		0
000134032	NA1		0003-1 PCI.	0713	0506			70.97		0
000134032	NA2		0015-2 PCI.	0784	0570			72.70		0
000134032	NA3		0075-3 PCI.	1407	0588			41.79		0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 625801			CONTRACT 22374-2104			PROJECT 02468			DATE - 10/27/76	
COMPOUND	TEST	ORG ID	DETECTOR TA1535	SPECIES	/	POP1	MUT1	FREQ1	CONTAM	
NAN			SOLVENT			EP+6	EP+0	EP-8		
NAP			FMS 0.2%			0081	4000	4938.27		
000134032	NA1		0003-1 PCT.			0230	0032	13.91	0	
000134032	NA2		0015-2 PCT.			0221	0043	19.46	0	
000134032	NA3		0075-3 PCT.			0274	0025	9.12	0	

REPORT EX33 LITTON HIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	ORG ID	CONTRACT	DETECTOR	TA1637	SPECIES	PROJECT	DATE
626501		22374-2104				02468	- 10/27/76
COMPOUND	TEST ID	CONCENTRATION					
NAN	SOLVENT		POPUP	MULTI		FREQ1	
NAP	AM 13 U6/ML		EP+6	EP+0		EP+8	
000134032	NA1	0003-1 PCI.	0221	0032	14.48	0	
000134032	NA2	0015-2 PCI.	0251	0240	95.62	0	
000134032	NA3	0075-3 PCI.	0162	0022	13.58	1	
			0208	0027	12.98	2	
			0132	0039	29.55	0	

REPORT EXP33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 626806 CONTRACT 22374-2104  
DETECTOR TA1538 SPECIES /  
PROJECT 02468 DATE - 10/27/76

COMPOUND	TEST ID	ORG	CONCENTRATION	POPUP	MUT1	FREQ1	CONTAM
NAN	SOLVENT		EP+6	EP+0		EP-8	
000134032	NA1	0003-1 PCT.	0155	0010	4.11	0	
					6.45	0	

REPORT EXR13 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 626402			CONTRACT 22374-2104	DETECTOR TA153B	SPECIES	PROJECT 02468	DATE - 10/27/76
COMPOUND	TEST	ORG ID	CONCENTRATION	POPUP	MUT1 EP+0	FREQ1 EP-0	CONTAM
NAN		SOLVENT	0911	0011	1.21	0	
NAP		NF 667 UG/ML	0410	0590	143.90	0	
000134032	NA1	0003-1 PCT.	0.384	0006	1.56	0	
000134032	NA2	0015-2 PCT.	0.454	0012	2.64	0	
000134032	NA3	0075-3 PCT.	0.600	0007	1.17	0	

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	TEST ID	SPECIES	PROJECT	DATE
628903	22374-2104 DETECTOR FA98		POPU EP+6	02468	- 10/27/76
COMPOUND	ORG ID	CONCENTRATION	MUTI EP+0	FREQ1 EP-B	CONTAM
NAN	SOLVENT	1000	0100	9.92	0
000134032	NA1	0003-1 PCT.	0712 . 0104	14.61	0

REPORT EXH33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 625905			CONTRACT 22374-2104 DETECTOR TAG#		SPECIES		PROJECT 02468		DATE - 10/27/76	
COMPOUND	TEST	ORG ID	CONCENTRATION		POPU	MUT1		FREQ1	EP-B	CONTAM
NAN			SOLVENT		0202	0028		13.86		0
NAP			NF 667 ug/ml		0049	0402		820.41		0
000134032	NA1		0003-1 PCT.		0120	0038		31.67		0
000134032	NA2		0015-2 PCT.		0283	0043		15.19		0
000134032	NA3		0075-3 PCT.		0300	0037		12.33		0
							:			

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 62BH01			PROJECT 0246B			DATE - 10/27/76		
COMPOUND	TEST ID	ORG CONCENTRATION	SPECIES	PROJECT	/	FREQ1	FREQ2	CONTAM
		EP+4	POP1	MUT1	EP+1	EP-5	EP-5	
NAN	SOLVENT	1118	0250	0109	22.36	9.75	0	
NAP	EMS 1.0 *	0294	0202	0112	68.71	38.10	0	
000134032	NA1 0005-0 PCT.	1216	0248	0297	20.39	24.42	0	
000134032	NA2 0025-1 PCT.	1160	0285	0125	24.57	10.78	0	
000134032	NA3 0125-2 PCT.	1270	0261	0146	20.55	11.50	0	

REPORT EXP33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104  
EXPERIMENT 627A05 DETECTOR TA100

DATE - 10/27/76

SPECIES ICRFLO/MOUSE

PROJECT 02468

COMPOUND	TEST	ORG	10	CONCENTRATION	POPU	MUL1	FREQ1	CONTAM
					EP+6	EP+0	EP-B	
A+C		DMN	90	UM/ML	2342	0482	20.58	0
A-C		SOLVENT			2274	0507	22.30	0
ALI		TISSUE			2604	0636	24.42	0
ALU		TISSUE			2104	0458	21.77	0
ACP	LI	DMN	90	UM/ML	1400	0982	70.14	0
ACP	LU	DMN	90	UM/ML	2506	0559	22.31	0
000134032	ACT	L11	0003-1	PCT.	2344	0545	23.25	0
000134032	ACT	L12	0015-2	PCT.	2028	0483	23.82	0
000134032	ACT	L13	0075-3	PCT.	2220	0554	24.95	0
000134032	ACT	L01	0003-1	PCT.	1962	0443	22.58	0
000134032	ACT	L02	0015-2	PCT.	1854	0438	23.62	0
000134032	ACT	L03	0075-3	PCT.	1952	0489	25.05	0

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 625904 CONTRACT 22374-2104  
DETECTOR TA1535 SPECIES ICRFLU/MOUSE

PROJECT 02468

DATE - 10/27/76

COMPOUND	TEST	OPG ID	CONCENTRATION	POPUL	MUTL	FREQ1 EP-6	CONTAM
A+C		DMN 90 UM/ML	0488	0037	7.58	0	
A-C		SOLVENT	0539	0030	5.57	0	
ALI		TISSUE	0357	0023	6.44	0	
ALU		TISSUE	0363	0029	7.57	0	
ACP	L1	DMN 90 UM/ML	0353	0481	136.26	0	
ACP	L0	DMN 90 UM/ML	0337	0091	27.00	0	
000134032	ACT	L11 0003-1 PCT.	0467	0037	7.92	0	
000134032	ACT	L12 0015-2 PCT.	0356	0037	10.39	0	
000134032	ACT	L13 0075-3 PCT.	0335	0049	14.63	0	
000134032	ACT	L01 0003-1 PCT.	0494	0028	5.67	0	
000134032	ACT	L02 0015-2 PCT.	0353	0020	5.67	0	
000134032	ACT	L03 0075-3 PCT.	0444	0058	13.06	0	

REPORT EXP33 LITTON RIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 629301			CONTRACT 22374-2104	DETECTOR TA1537	SPECIES ICRFLO/MOUSE	PROJECT 02468	DATE - 10/27/76
COMPOUND	TEST ID	ORG	CONCENTRATION	POPUP	MUT1	FREQ1	CONTAM
A+C		AMQ	333 UG/ML	0595	0016	2.69	0
A-C		SOLVENT		0547	0032	5.05	0
ALI		TISSUE		0510	0036	6.95	2
ALU		TISSUE		0548	0020	3.65	0
ACP	LI	AMQ	333 UG/ML	0311	0444	142.77	0
ACP	LU	AMQ	333 UG/ML	0608	0013	2.14	0
000134032	ACT	LI1	0003-1 PCT.	0648	0051	7.87	0
000134032	ACT	LI2	0015-2 PCT.	0448	0043	9.60	0
000134032	ACT	LI3	0075-3 PCT.	0586	0038	6.48	0
000134032	ACT	LU1	0003-1 PCT.	0646	0047	7.28	0
000134032	ACT	LU2	0015-2 PCT.	0574	0047	8.19	0
000134032	ACT	LU3	0075-3 PCT.	0646	0051	7.89	0

REPORT EXP33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 626001 CONTRACT 22374-2104 PROJECT 02468  
DETECTOR TA1538 SPECIES ICRFL0/MOUSE DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUT1	FREQ1	CONTAM
				EP+6	EP+0	EP-8	
A+C		ANTH 67	UG/ML	0954	0277	29.04	0
A-C		SOLVENT		0862	0246	28.54	0
ALI		TISSUE		0523	0260	49.71	0
ALU		TISSUE		0924	0236	25.54	0
ACP	L1	ANTH 67	UG/ML	0449	0910	202.67	0
ACP	LU	ANTH 67	UG/ML	0842	0323	38.36	0
000134032	ACT	L11	0003-1 PCT.	2005	0271	13.52	0
000134032	ACT	L12	0015-2 PCT.	0748	0185	24.73	0
000134032	ACT	L13	0075-3 PCT.	0517	0176	34.04	0
000134032	ACT	L01	0003-1 PCT.	0953	0155	16.26	0
000134032	ACT	L02	0015-2 PCT.	0643	0154	23.95	0
000134032	ACT	L03	0075-3 PCT.	0851	0186	21.86	0

REPORT EXR33 LITTON-BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104  
EXPERIMENT 628904 DETECTOR TA98

PROJECT 02468  
SPECIES ICARFL0/MOUSE

DATE - 10/27/76

COMPOUND	TEST ID	ORG	ORG ID	CONCENTRATION	POPUP	MUT1	FREQ1	CONTAM
ALU		TISSUE		1225	0106	8.65	2	
000134032	ACT	LU1	0003-1	PCI.	0814	0133	16.34	0

REPORT EXP33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 627206 CONTRACT 22374-2104  
DETECTOR TA98 SPECIES ICRFLO/MOUSE

PROJECT 02468

DATE - 10/27/76

COMPOUND	TEST ID	ORG ID	CONCENTRATION	P0PU	MUT1	FREQ1	CONTAM
			UG/ML	EP+6	EP+0	EP-8	
A+C		ANTH	67 UG/ML	0657	0031	4.072	0
A-C		SOLVENT		1003	0031	3.009	0
ALI		TISSUE		0714	0071	9.94	0
ALU		TISSUE		0702	0038	5.041	0
ACP	LI	ANTH	67 UG/ML	0603	0641	106.30	0
ACP	LU	ANTH	67 UG/ML	0770	0850	110.39	0
000134032	ACT	L11	0003-1 PCT.	0421	0041	9.74	0
000134032	ACT	L12	0015-2 PCT.	0289	0044	15.022	0
000134032	ACT	L13	0075-3 PCT.	0326	0064	19.63	0
000134032	ACT	L01	0003-1 PCT.	0264	0123	46.59	0
000134032	ACT	L02	0015-2 PCT.	0439	0058	13.21	0
000134032	ACT	L03	0075-3 PCT.	0468	0063	13.46	0

REPORT EXH33 LITTON AIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 629202 CONTRACT 22374-2104  
DETECTOR 000004 SPECIES ICRFLO/MOUSE  
PROJECT 02468 DATE - 10/21/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
A+C		DMN 90 UM/ML	1474	0343	0120	23.27	0.14	0	
A-C		SOLVENT	1304	0341	0104	26.15	7.98	0	
ALI		TISSUE	1244	0330	0108	26.53	8.68	0	
ALU		TISSUE	1194	0345	0103	28.89	8.63	0	
ACP	LJ	DMN 90 UM/ML	0843	0574	0232	67.69	27.36	0	
ACP	LU	DMN 90 UM/ML	1111	0360	0127	32.40	11.43	0	
000134032	ACT	L11 0005-0 PCI.	1116	0241	0127	21.59	11.38	0	
000134032	ACT	L12 0025-1 PCI.	1128	0225	0094	19.95	8.33	0	
000134032	ACT	L13 0125-2 PCI.	1280	0342	0094	26.72	7.34	0	
000134032	ACT	L01 0005-0 PCI.	1130	0329	0134	29.12	11.86	0	
000134032	ACT	L02 0025-1 PCI.	1098	0286	0094	26.05	8.56	0	
000134032	ACT	L03 0125-2 PCI.	1012	0282	0099	27.87	9.78	0	

REPORT EX433 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 627801 CONTRACT 22374-2104  
DETECTOR 1A100 SPECIES SPRDAW/RAT

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUTI	FREQ1	CONTAM
A+C		DMN 90 UM/ML	2500	0518	EP+0	20.72	0
A-C		SOLVENT	2288	0591		25.83	0
ALI		TISSUE	2172	0683		31.45	0
ALU		TISSUE	2008	0543		27.04	0
ACP	LI	DMN 90 UM/ML	1419	0876		61.73	0
ACP	LU	DMN 90 UM/ML	2254	0636		28.22	0
000134032	ACT	L11 0003-1 PCI.	2250	0719		31.96	0
000134032	ACT	L12 0015-2 PCI.	2146	0860		40.07	0
000134032	ACT	L13 0075-3 PCI.	2402	0860		35.80	0
000134032	ACT	L01 0003-1 PCI.	1062	0617		58.10	0
000134032	ACT	L02 0015-2 PCI.	1956	0640		32.72	0
000134032	ACT	L03 0075-3/PCI.	1968	0683		34.71	0

REPORT EXP33 LITTON RIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104  
EXPERIMENT 626701 DETECTOR TA1535 SPECIES SPRAEW/RAT

PROJECT 02468

DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUP	MUT1 EP+6	FREQ1 EP+8	CONTAM
A+C		DMN 90 UM/ML	0909	0098	10.78	0	
A-C	SOLVENT		1063	0108	10.16	0	
ALI	TISSUE		0672	0091	13.54	0	
ALU	TISSUE		0823	0104	12.64	2	
ACP	L1	DMN 90 UM/ML	1272	3140	246.86	0	
ACP	LU	DMN 90 UM/ML	0681	0096	13.97	2	
000134032	ACT	L11 0003-1 PCT.	0570	0093	16.32	2	
000134032	ACT	L12 0015-2 PCT.	0814	0125	15.36	2	
000134032	ACT	L13 0075-3 PCT.	0640	0083	12.97	2	
000134032	ACT	L01 0003-1 PCT.	1417	0079	5.58	0	
000134032	ACT	L02 0015-2 PCT.	1504	0067	4.45	2	
000134032	ACT	L03 0075-3 PCT.	1297	0078	6.01	0	

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 629501 CONTRACT 22374-2104  
PROJECT 02468 DATE - 10/27/76  
DETECTOR TA1537 SPECIES SPRDAW/RAT

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUTL	FREQ1	CONTAM
				EP+6	EP+0	EP-8	
A+C		AMQ 333 UG/ML	0600	0025		4.17	0
A-C	SOLVENT		0641	0017		2.65	0
ALI	TISSUE		0669	0025		3.74	1
ALU	TISSUE		0568	0012		2.11	0
ACP	LI	AMQ 333 UG/ML	0200	0245		122.50	1
ACP	LU	AMQ 333 UG/ML	0582	0008		1.37	2
000134032	ACT	L11 0003-1 PCI.	0569	0017		2.99	0
000134032	ACT	L12 0015-2 PCI.	0592	0016		2.70	0
000134032	ACT	L13 0075-3 PCI.	0585	0017		2.91	0
000134032	ACT	L01 0003-1 PCI.	0590	0012		2.03	1
000134032	ACT	L02 0015-2 PCI.	0574	0012		2.09	1
000134032	ACT	L03 0075-3 PCI.	0618	0012		1.94	1

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104  
EXPERIMENT 626505 DETECTION TA1538 SPECIES SPRDAW/RAT  
PROJECT 02468

COMPOUND	TEST	ORG ID	CONCENTRATION	POPNU	MUTU	FREQ1 EP-8	CONTAM
A+C		ANTH 67	UG/ML	0427	0021	4.92	0
A-C		SOLVENT		0486	0020	4.12	0
ALI		TISSUE		0271	0050	18.45	0
ALU		TISSUE		0374	0028	7.49	0
ACP	LI	ANTH 67	UG/ML	0269	0588	218.59	0
ACP	LU	ANTH 67	UG/ML	0250	0683	273.20	0
000134032	ACT	LI1	0003-1 PCT.	0183	0027	14.75	0
000134032	ACT	LI2	0015-2 PCT.	0166	0028	16.87	0
000134032	ACT	LI3	0075-3 PCT.	0344	0024	6.98	0
000134032	ACT	LU1	0003-1 PCT.	0186	0031	16.67	0
000134032	ACT	LU2	0015-2 PCT.	0252	0033	13.10	0
000134032	ACT	LU3	0075-3 PCT.	0292	0024	8.22	0

REPORT EXR33 LITTON RIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	SPECIES	PROJECT	DATE	
626502	22374-2104 DETECTOR TA9H	SPRDRAW/RAT	02468	- 10/27/76	
COMPOUND	TEST ID	ORG CONCENTRATION	MUT1 EP+0	FREQ1 EP-B	
				CONTAM	
A+C	ANTH 67	06/ML	1712 0272	15.89	0
A-C	SOLVENT		2147 0267	12.44	0
ALI	TISSUE		2557 0297	11.62	0
ALU	TISSUE		1650 0331	20.06	0
ACP	LI	ANTH 67 06/ML	1116 0938	84.05	0
ACP	LU	ANTH 67 06/ML	1304 0324	24.85	0
000134032	ACT	LI1 0003-1 PCT.	1015 0224	22.07	0
000134032	ACT	LI2 0015-2 PCT.	0885 0231	26.10	0
000134032	ACT	LI3 0075-3 PCT.	0940 0229	24.36	0
000134032	ACT	LU1 0003-1 PCT.	1308 0357	27.29	0
000134032	ACT	LU2 0015-2 PCT.	1468 0280	19.07	0
000134032	ACT	LU3 0075-3 PCT.	1394 0279	20.01	0

REPORT EXP33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 629502 CONTRACT 22374-2104 DETECTION 0000014 SPECIES SPRDAW/RAT PROJECT 02468 DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUP EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
A+C		DMN 90 UM/ML	0086	0381	0191	43.00	21.56	0	
A-C	SOLVENT		0761	0390	0155	51.25	20.37	0	
ALI	TISSUE		0708	0296	0187	41.81	26.41	0	
ALU	TISSUE		0726	0284	0153	39.12	21.07	0	
ACP	L.I	DMN 90 UM/ML	0567	0453	0343	79.89	60.49	0	
ACP	L.U	DMN 90 UM/ML	0626	0338	0186	40.92	22.52	0	
000134032	ACT	L11 0005-0 PCT.	0837	0321	0124	38.35	14.81	0	
000134032	ACT	L12 0025-1 PCT.	0750	0324	0133	43.20	17.73	0	
000134032	ACT	L13 0125-2 PCT.	0611	0300	0134	49.10	21.93	0	
000134032	ACT	L01 0005-0 PCT.	0754	0298	0103	39.52	13.66	0	
000134032	ACT	L02 0025-1 PCT.	0712	0293	0101	41.15	14.19	0	
000134032	ACT	L03 0125-2 PCT.	0573	0279	0091	48.69	15.88	0	

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 627901 CONTRACT 22374-2104 DETECTOR TA100 SPECIES RHECUS/MONKEY PROJECT 02468

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUP	MUT1	FREQ1	CONTAM
A+C		DMN 90 UM/ML	1736	0464	26.73	0	
A-C		SOLVENT	1842	0476	25.84	0	
ALI		TISSUE	2708	0814	30.06	0	
ALU		TISSUE	2442	0699	28.62	0	
ACP	L1	DMN 90 UM/ML	1184	0713	60.22	0	
ACP	LU	DMN 90 UM/ML	2430	0752	30.95	0	
000134032	ACT	L11 0003-1 PCI.	2344	0707	30.16	0	
000134032	ACT	L12 0015-2 PCI.	1932	0619	32.04	0	
000134032	ACT	L13 0075-3 PCI.	2454	0650	26.49	0	
000134032	ACT	L01 0003-1 PCI.	2148	0664	30.91	0	
000134032	ACT	L02 0015-2 PCI.	2312	0618	26.73	0	
000134032	ACT	L03 0075-3 PCI.	2570	0748	29.11	0	

REPORT EXP33 LITTON AIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 62A001 CONTRACT 22374-2104  
DETECTOR TA1535 SPECIES RHECUS/MONKEY

PROJECT 02468  
DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUP	MUT1	FREQ1	CONTAM
			EP/ML	EP+6	EP+0	EP-B	
A+C		DMN 90	UM/ML	2201	0164	7.45	0
A-C		SOLVENT		2313	0223	9.64	0
ALI		TISSUE		2157	0190	6.89	0
ALU		TISSUE		2683	0203	7.57	0
ACP	LI	DMN 90	UM/ML	1754	1020	58.15	0
ACP	LU	DMN 90	UM/ML	2777	0245	8.82	0
000134032	ACT	LI1	0003-1 PCT.	2400	0185	7.71	0
000134032	ACT	LI2	0015-2 PCT.	2493	0240	9.63	0
000134032	ACT	LI3	0075-3 PCT.	2219	0169	7.62	0
000134032	ACT	LU1	0003-1 PCT.	2816	0195	6.92	0
000134032	ACT	LU2	0015-2 PCT.	2469	0196	7.94	0
000134032	ACT	LU3	0075-3 PCT.	2870	0196	6.83	0

REPORT EXP33 LITTON RIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 629701 CONTRACT 22374-2104  
DETECTOR TA1537 SPECIES Rhesus/Monkey

PROJECT 02468  
DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUP	MUT1	FREQ1	CONTAM
			UG/ML	EP+6	EP+0	EP-8	
A+C		AMQ 333	UG/ML	2774	0327	11.79	2
A-C		SOLVENT		1818	0145	7.98	2
ALI		TISSUE		0981	0101	18.45	2
ALU		TISSUE		0996	0221	22.19	2
ACP	L1	AMQ 333	UG/ML	2119	0065	3.07	2
ACP	LU	AMQ 333	UG/ML	2400	0315	13.13	2
000134032	ACT	L11	0003-1 PCT.	0841	0165	19.62	0
000134032	ACT	L12	0015-2 PCT.	0787	0161	20.46	0
000134032	ACT	L13	0075-3 PCT.	0896	0177	19.75	0
000134032	ACT	LU1	0003-1 PCT.	1087	0266	24.47	0
000134032	ACT	LU2	0015-2 PCT.	0739	0337	45.60	0
000134032	ACT	LU3	0075-3 PCT.	1146	0269	23.47	0

REPORT EXP33 LITTON RIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104  
EXPERIMENT 627104 DETECTOR TA1538 SPECIES RHESUS/MONKEY

PROJECT 92468

DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUP	MUT1	FREQ1	CONTAM
			EP/ML	EP+6	EP+0	EP-8	
A+C		ANTH 67	UG/ML	0544	0018	3.31	0
A-C		SOLVENT		0306	0019	6.21	0
ALI		TISSUE		0418	0045	10.77	0
ALU		TISSUE		0554	0021	3.79	0
ACP	L1	ANTH 67	UG/ML	0334	1940	580.84	0
ACP	LU	ANTH 67	UG/ML	0674	0026	3.86	0
000134032	ACT	L11	0003-1 PCT.	0194	0023	11.86	0
000134032	ACT	L12	0015-2 PCT.	0198	0009	4.55	0
000134032	ACT	L13	0075-3 PCT.	0194	0020	10.31	0
000134032	ACT	L01	0003-1 PCT.	0110	0019	17.27	0
000134032	ACT	L02	0015-2 PCT.	0080	0012	15.00	0
000134032	ACT	L03	0075-3 PCT.	0180	0009	5.00	0

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 627401 CONTRACT 22374-2104 DETECTOR TA98 SPECIES RHESUS/MONKEY PROJECT 02468 DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUTL	FREQ1	CONTAM
				EP+6	EP+0	EP-B	
A+C		ANTH 67	UG/ML	1559	0412	26.43	1
A-C		SOLVENT		1803	0489	27.12	0
ALI		TISSUE		0916	0518	56.55	0
ALU		TISSUE		0753	0463	61.49	1
ACP	LI	ANTH 67	UG/ML	1088	0963	88.51	1
ACP	LU	ANTH 67	UG/ML	0953	0409	42.92	0
000134032	ACT	L11	0003-1 PCT.	0982	0403	41.04	1
000134032	ACT	L12	0015-2 PCT.	1362	0374	27.46	0
000134032	ACT	L13	0075-3 PCT.	1235	0382	30.93	0
000134032	ACT	L01	0003-1 PCT.	1185	0470	39.66	1
000134032	ACT	L02	0015-2 PCT.	1069	0443	41.44	0
000134032	ACT	L03	0075-3 PCT.	1229	0429	34.91	0

REPORT EXH33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 629503 CONTRACT 22374-2104  
DETECTOR 000004 SPECIES RHECUS/MONKEY  
PROJECT 02468

DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUP EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
A+C		DNN 90 UM/ML	0787	0104	0059	13.21	7.50	0	
A-C		SOLVENT	0624	0101	0047	16.19	7.53	0	
ALI		TISSUE	0751	0118	0050	15.71	7.72	0	
ALU		TISSUE	0734	0064	0042	8.72	5.72	0	
ACP	L1	DNN 90 UM/ML	0721	0486	0177	67.41	24.55	0	
ACP	LU	DNN 90 UM/ML	0735	0095	0045	12.93	6.12	0	
000134032	ACT	L11 0005-0 PCI.	0798	0118	0053	14.79	6.64	0	
000134032	ACT	L12 0025-1 PCI.	0672	0113	0049	16.82	7.29	0	
000134032	ACT	L13 0125-2 PCI.	0736	0102	0044	13.86	5.98	0	
000134032	ACT	LU1 0005-0 PCI.	0734	0089	0039	12.13	5.31	0	
000134032	ACT	LU2 0025-1 PCI.	0728	0086	0045	11.81	6.18	0	
000134032	ACT	LU3 0125-2 PCI.	0790	0090	0061	11.39	7.72	0	